

Mitigation of Salinity Stress in Maize by Silicon Nutrition

By

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2005-ag-1919

M.Sc. (Hons.) Agriculture

(Soil Science)

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR THE
DEGREE OF**

DOCTOR OF PHILOSOPHY

IN

SOIL SCIENCE



INSTITUTE OF SOIL & ENVIRONMENTAL SCIENCES

Faculty of Agriculture, University of Agriculture, Faisalabad, Pakistan

2015

To,

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Waqas Ud Din Khan

DEDICATED TO MY DEAR PARENTS*

**THEIR EAGERNESS FOR MY HIGHER EDUCATION
HAS TRULY BRIGHTENED MY LIFE**

ACKNOWLEDGEMENTS

In the name of **Almighty ALLAH**, the most Gracious and Merciful. To Almighty Allah we pray that He may guide us the right path, crown our endeavors with success, and bless our lives with abundant prosperity. Countless Darood-o-Salam upon the Lovingly **Holy Prophet MUHAMMAD (Peace Be Upon Him)**, the fountains of knowledge, who has guided his “Ummah” to seek knowledge from cradle to grave.

I feel highly privileged to express my heartfelt gratitude to my supervisor **Dr. Tariq Aziz**, Associate Professor of Soil Science, for his guidance and helpful suggestions during the research work and for critically reviewing the manuscript. His kind and generous response to my difficulties during the whole course of degree will never be forgotten.

I offer my sincere thanks to the rest of my thesis committee members: Prof. Dr. Muhammad Khalid and Dr. Ejaz A. Warraich. Their valuable feedback helped me to improve the dissertation in many ways.

I am indebted to my mentor Dr. Thomas G. Reichenauer and Dr. M. Naveed for providing guidelines about research planning. I am also thankful to my some other lab fellows, Miss Mahreen Gul, Ch. Hasseb Arshad, Hafiz M. Bilal, P. M. adnan Ramzani, Shahbaz Ali, Abdul Haseeb, M. Asad Bashir and especially our lab attendant Mr. Akhter who cooperated with me well as and when required. At the end, I regret if I have forgotten any name.

No acknowledgement could ever efficiently express my obligations to my affectionate and adoring parents, affectionate sister and helping brother whose hands always been raised in prayers for me. Without their moral and financial support, the present distinction would have been merely a dream. They always acted as torch bearer for me in the dark valleys of mortal life.

It is a matter of great pleasure and honor for me to express my gratitude and appreciation to my wife and daughter for their kind support and encouragement.

May ALLAH bless all those people with long peaceful and happy life.

Waqas Ud Din Khan

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Abstract

Salinity stress is a serious threat to crop production around the globe and about 6.3 m ha lands in Pakistan is salt affected. Maize is an important cereal crop having significant impact on Pakistan's economy and food security. Being glycophyte, maize yield is seriously affected if grown on salt affected soils. Nutrition management can improve salinity tolerance in crop. Silicon (Si) is a beneficial element having many beneficial effects, such as improving water use efficiency and enhancement of salt tolerance. We hypothesized that silicon nutrition management can increase growth, yield and production of maize crop under salt stress conditions. To explore the role of Si in alleviating salinity stress in maize and to identify the mechanism responsible for improved growth, a project was proposed with six independent studies to screen maize germplasm for salinity tolerance and response of selected genotypes to applied Si under salinity stress. Various growth and physiological parameters were studied in petri plates, pots, hydroponics and field experiments. The attributes related to maize germination and early vegetative growths were significantly reduced by salinity stress while Si application improved germination parameters and ionic concentration under salt stress. Then vegetative growth was tested in hydroponics where the toxic effects of salt stress on different physiological (chlorophyll contents, chlorophyll fluorescence, gaseous exchange), ionic (Na, K concentration in shoots and roots) and biochemical (enzyme activity assays and phenolic contents) parameters were studied, while Si application minimized both osmotic and oxidative stresses under salt stress. In the field study, grain yield, straw yield, harvest index, number of grains per cob and other parameters related to maize crop production were evaluated in saline and non-saline conditions. Cultivars showed significant genotypic variation and foliar Si application suggested to be a viable strategy for maize growth under saline and non-saline fields. This study implies that Si application could enhance maize growth on every growth stage by manipulating the deleterious effects of salinity.

Introduction

Food security is a serious threat in developing countries because of ever-increasing population. According to recent estimates, more than 2 billion people face food shortage occasionally due to poverty and/or natural calamities (FAO, 2009). The problem can be tackled by: a) efficient utilization of the natural resources b) proper application of physical inputs c) increasing production of major food crops through either increasing area under crop production or improving crop yields per hectare. To increase area under crop production is not feasible due to limited farmer resources and poor management. The area available for agriculture is decreasing day by day due to: a) urbanization of arable land b) poor soil management practices with intensive use of cultivation (Gruhn et al., 2000) c) degradation of the existing arable land due to various abiotic factors including salinity and drought (Cakmak, 2002). Hence there is dire need to improve crop yields per hectare and to take more and more poor or marginal soils under cultivation.

One of the main reasons of soil desertification is soil salinity. Out of 13 billion hectares of total land, one billion is salt affected, including 30% of all irrigated land (Rengasamy, 2006). Most of this salt affected land is not under cultivation and has very low productivity, if cultivated. Hence, for sustaining food security, a high priority should be given to safe use of salt affected soils. Maize is a major cereal crop of Pakistan growing on a large area of 1168 (000 ha), and its production is 4944,000 tonnes (GOP, 2015) and has a significant potential for securing food availability. In Pakistan, 6.3 mha land is salt affected (Alam et al., 2000); if this salt affected area is brought under maize cultivation, additionally 13.4 m tons of maize production would be expected, considering 50% yield reduction in these poor soils.

Soil salinity has a number of deleterious effects on crop growth such as ion toxicity and physiological drought, decrease in water use efficiency and photosynthesis due to interveinal chlorosis which ultimately decreases crop yields (Munns et al., 2006; Nasim et al., 2008; Tahir et al., 2012). Salt stress is also the major reason to cause imbalance of the inner cellular ions (Chung et al., 2008; Nasim et al., 2008). The major reason of reduced growth of cereal crops in

salt stressed condition is specific ion toxicity (certain ions like Na and Cl uptake at elevated level) (Chinnusamy et al., 2005; Tahir et al., 2011; 2012). More than 50% yield reduction in maize has been reported in soils with EC more than 6 (USDA, 2011).

Taking salt affected soils under cultivation and improving crop yields on salt affected soils would be beneficial for ensuring food security. It is highly recommended to adopt strategies aiming at utilization of these marginal lands and increased crop production on salt affected lands (Irrigation water, raising beds, organic matter and salt tolerant crops like kallar grass). All of these strategies have some advantages and disadvantages and are well reported. Judicious use of mineral nutrition is a recommended shotgun strategy as it strengthens the plants to cope against salt stress. Silicon (Si) as a beneficial nutrient is known to improve plant growth particularly under abiotic stresses. It is helpful for plants in many ways as it improves plant water status in context of relative water content and transpiration rate (Romero-Aranda et al., 2006), ameliorates the harmful effects of salinity on chlorophyll content and plant biomass (Tuna et al., 2008) in both leaves and roots, it lowers significantly the Na⁺ concentrations (Kafi and Rahimi, 2011; Tahir et al., 2012).

Salinity-silicon interactions have been investigated in a number of plant species (Al-Aghabary et al., 2004; Zhu et al., 2004; Liang et al., 2005; Tahir et al., 2012). Increasing the availability of Si in the growth medium can reduce salinity stress in plants by altering soil and plant factors (Kafi and Rahimi, 2011), but specific mechanisms are still debatable. Liang et al. (2007) reviewed that silicon uptake in a salt stressed plant increases root activity for nutrient uptake, inhibits transpiration which reduces osmotic stress. Similarly, Si was found to increase the total dry matter, relative water content, and chlorophyll content in wheat (Tahir et al., 2012). Additionally, it decreases electrolyte leakage and proline accumulation in maize plants. It also increases the activity of ATPase & PPase in plasma membrane which ultimately increases K and decrease Na uptake (Tuna et al., 2008).

The beneficial effects may vary among plant species. Cereals that accumulate maximum Si in their shoots usually performed better than the others (Ma et al. 2001a). Rice is a typical example, which accumulates up to 10% Si on a dry weight basis in the shoot. Higher contents of Si in rice have been revealed to be essential for healthy growth, stable yield and production. For this reason, Si has been acknowledged as an "agronomically essential element" in Japan and silicate

fertilizers have been applied to paddy soils (Ma, 2004); but Si salt is not a cheap source to apply in field conditions alone as a soil amendment, so different methods like soil and foliar Si applications are used to make it economically viable strategy. Foliar Si application has already been used to combat heavy metal toxicity like cadmium in pots (Liu et al., 2009). The other typical beneficial effects of Si are usually expressed more clearly when plants are subjected to various abiotic and biotic stresses (Ma, 2004). Silicon is probably the only element which is able to enhance the resistance to multiple stresses.

Some work has been conducted in Pakistan on Si nutrition of agronomic crops (Ashraf et al., 2012; Tahir et al., 2012; Tahir et al., 2011), however role of Silicon in salinity tolerance of maize has not been explored in detail and only one report is available of a hydroponic study (Perveen and Ashraf, 2012). There is no report on effect of Si on germination, early vegetative, vegetative and reproductive stages, as plants respond differently to salinity at different growth stages. A cultivar sensitive to salinity at germination, may have more tolerance at vegetative growth stage as effect of salinity varies at both stages. At germination and early vegetative growth stages, the main effect of salinity is physiological drought (Munns, 2006), and at later growth stages, ionic toxicity is the main deleterious factor for decreased crop growth. The present research project was designed first to screen maize genotypes at germination and at early vegetative growth stages and to evaluate the role of silicon on different growth stages of salt stressed maize cultivars. The selected maize cultivars were then grown with and without Si application at different growth stages to study the salinity tolerance mechanisms as influenced by Si application. Finally, different Si application methods were evaluated at field conditions in a confirmation experiment of previous results.

Review of literature

2.1 Salinity stress: A general overview

Soil salinity is detrimental in plant life as negatively affecting metabolic and physiological processes eventually reducing growth and yield of agronomic crops (Ashraf and Harris, 2004). Salinity stress induces specific changes in physiological, morphological and metabolic processes like seed germination, seedling growth and vigour, flowering, fruit set, activities of enzymes, integrity of cellular membrane, and the functioning of the plant photosynthetic apparatus (Sairam and Tyagi, 2004).

Excess amount of salts present in the soil solution have an adverse effect on plant growth and development. Primary minerals in the exposed layer of earth crust or weathering of rocks are the major and primary source of salinity in the soils. Nearly 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity (Zhu, 2001). It includes approximately 200 million hectares in Americas, large portion of eastern and southern Europe, and 120 million ha in the Middle East, 80 million ha in the Africa, 35 million ha in Asia and over 6 million ha in Australia. In Pakistan, the salt-affected soils are mainly confined to arid- semi arid plains and are estimated to be 6.30 Mha (Alam et al., 2000). Out of these, 1.89 Mha are saline, 1.85 Mha permeable saline-sodic, 1.02 Mha impermeable saline-sodic, and 0.028 Mha sodic in nature. Soil salinity proves to be more detrimental relative to saline irrigation water (Figure 2.1). In Cereal crops, barley and wheat are semi tolerant to applied salinity as 50 % yield reduction was reported on ECe 18 and 13 dS/m, respectively. However, rice and maize shown 50 % yield reduction on ECe 7.2 and 5.9 dS/m, respectively (Figure 2.1).

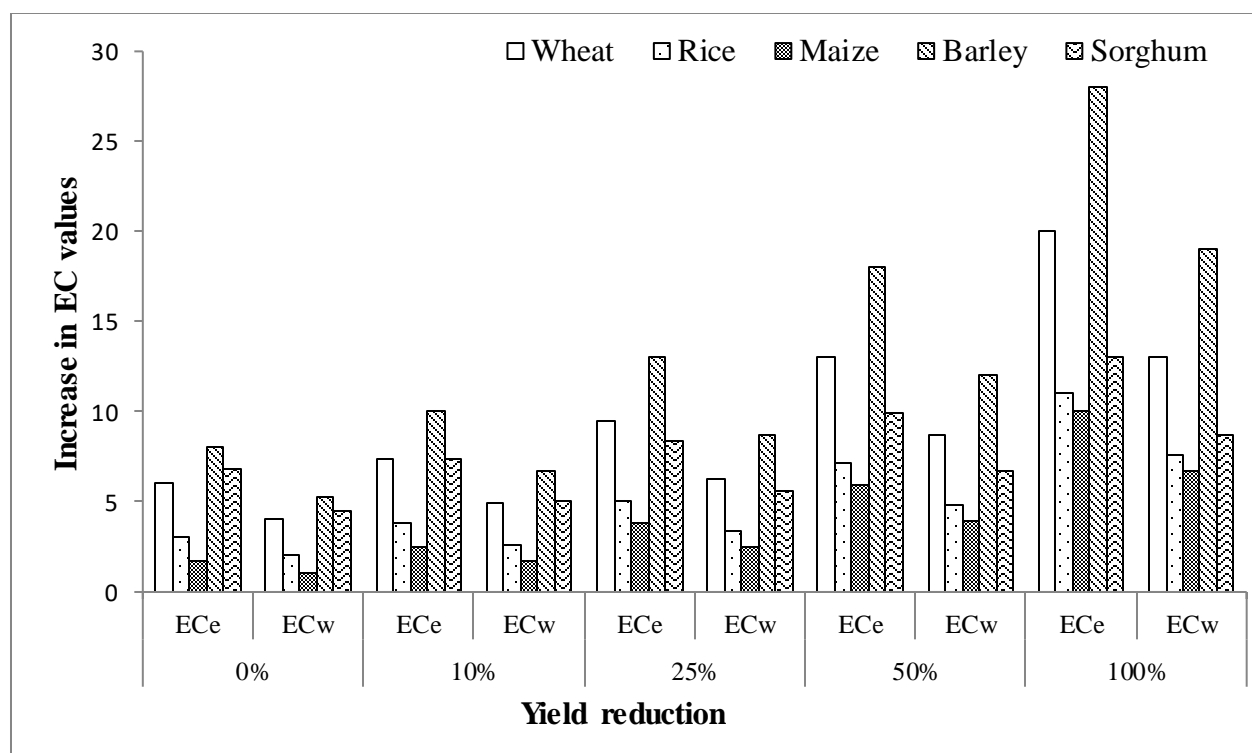


Figure 2.1 Yield reduction and crop tolerance by cereal crops as influenced by the soil salinity (ECe) and irrigation water salinity (ECw), (modified from USDA, 2011).

2.2 Detrimental Effects of Salinity on Crop Growth

Salt stress reduces the growth and development of cereal crops; however the reduction in crop yield varies with the growth stage and degree of applied stress. Barley and wheat are sensitive to salinity stress in seedling growth stages and ECe must remain below 4 dS/m during initial stages of growth (USDA, 2011). The effects of salt stress on germination, different vegetative growth stages, physiology, production and yield of cereal crops are concisely discussed below.

2.2.1 Effects on Germination

Seed germination is the most important phase of seedling growth as it determines the success of seedling establishment and crop growth. Seed is called germinated when both radical and plumule germinate upto 2 mm. The whole seed germination period includes imbibition, protrusion, germination and seedling establishment stages. There are two distinguished metabolic

processes involved in seed germination: (1) new cell formation, and (2) enzymatic hydrolysis of seed storage (Copeland and McDonald, 1985). Formation of different seedling tissues in cereals involved different steps. Initially gibberellic acid (GA) is synthesized in the scutellum and later on it is transferred to the aleurone layer where it is involved in the synthesis of hydrolytic enzymes (Figure 2.2); responsible for hydrolysis of different substrates being utilized in synthesis of different seedling tissues (Soltani et al., 2012)

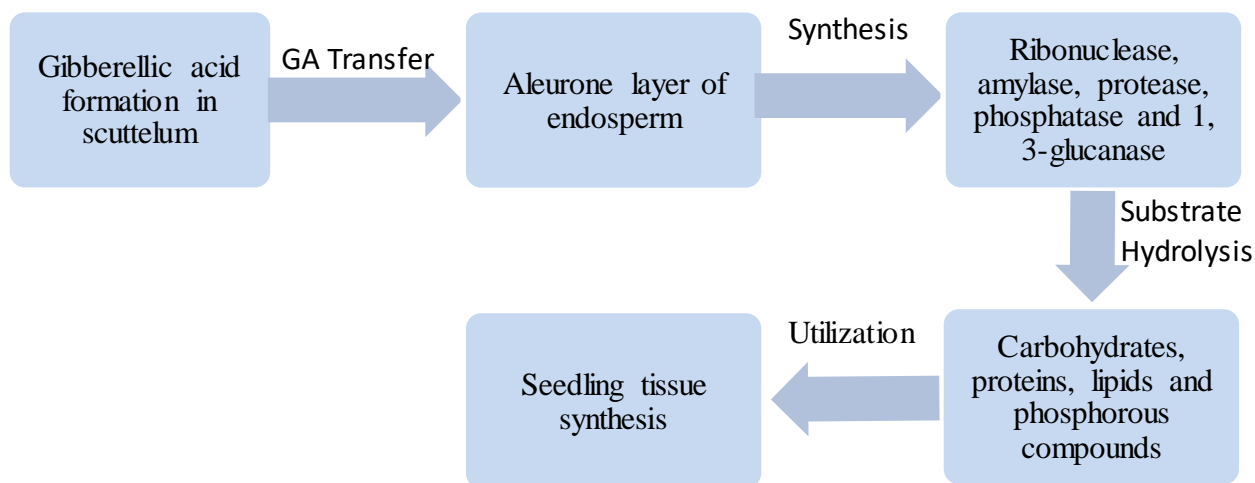


Figure 2.2 Schematic diagram of processes from seed germination to seedling tissue synthesis (reviewed from Soltani et al., 2012)

This germination process requires favorable environment and any change in environment like abiotic stress including salinity hinders this process. Salinity reduces the rate of germination events and delays the onset (Ashraf and Foolad, 2005). It ultimately leads to the reduced plant growth and lower crop yield; as early germination stage (0–5 days) was found to be the salt sensitive stage in rice (Zhou-fei et al., 2010).

Radical and plumule length are important traits in germination stages regarding salinity stress and significant reduction in both these parameters have been reported under salinity stress (Janmohammadi et al., 2008). An obvious reduction was reported in radicle, plumule and seedling length in different maize varieties subjected to salt stress (Farsiani and Ghobadi, 2009; Khayatnezhad et al., 2010); along with a significant reduction in seed vigor and germination index (Janmohammadi et al., 2008). Germination percentage decreases with increasing level of

NaCl as mean germination time increases (Khodarahmpour, 2012). Reduction in germination percentage because of salinity stress has also been reported in pearl millet (Ashraf et al., 1999) and in barley (Hussain et al., 1997). This low germination is related to salinity induced disturbance of metabolic processes leading to increase in plant secondary metabolites like phenolic compounds (Ayaz et al., 2000).

Seed vigor is an important trait regarding germination of cereals; it decreases with increase in concentration of NaCl in soil solution. Seed vigor increases in osmotic potential until -3 bar but decreased in -5 bar and there were no germination and growth measured in all genotypes at high salinity levels -15 bar (Mostafavi, 2011).

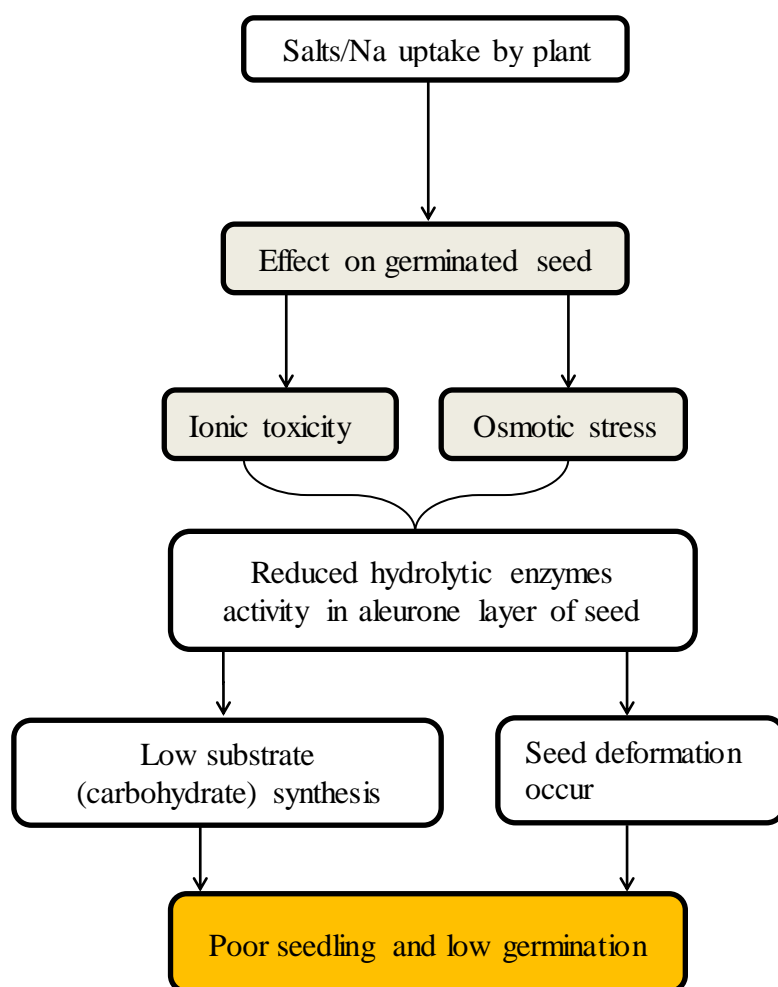


Figure. 2.3 Schematic diagram of salt stress effects on cereal germination (Reviewed from Ashraf and Foolad, 2005)

2.2.2 Early vegetative growth

Effects of salinity stress are variable at different growth stages and plants respond quite differently to various salt treatments. Nuran and Cakirlar (2002) reported that at varying levels of salt, germination started and seeds were emerged, but their development could not be continued. In wheat and barley, the seedling or early vegetative growth stage is known to be more sensitive to salt stress compared with later growth stages (Bhutta and Hanif, 2010; Khayatnezhad and Gholamin, 2010). Maize, a glycophyte crop was also reported as sensitive at early growth stages but tolerant at later stages (Khatoon et al., 2010). In barley, early vegetative growth started from day 6 to day 20 while in wheat it started from day 13 to day 20 (Katerji et al., 2009). In wheat and barley, there was a reduction in leaf size and plants height due to salt stress. Elevated rate of salinity @ 80 and 160 mM affected shoot growth more severely than root growth of wheat seedlings (El-Hendawy et al., 2011).

Salinity stress causes a significant decrease in shoot fresh and dry weights, its length, and leaf area (Bhutta and Hanif, 2010). Similarly Grewal, (2010) reported that symptoms of salt stress started appearing two weeks after sowing in wheat and barley particularly at highest level of subsoil NaCl salinity.

2.2.3 Vegetative growth

Salinity stress causes severe problems in cereals also at vegetative stage similar to those observed under early vegetative growth. Under saline conditions, elevated Na^+ concentration hampered the growth and development of plants as it suppresses the photo-system II photochemical activity and leaf gas exchange (Dionisio-Sese and Tobita, 2000a).

Rice is a salt sensitive cereal crop and at EC 8-10 dSm^{-1} , most rice plants are completely damaged (Islam et al., 2007) and 30-50% yield reduction has been reported in rice (Islam et al., 2007).

Wheat genotypes differ significantly in salinity tolerance (Munns, 2002; Flowers, 2004; Saqib et al., 2005; Tahir et al., 2012). Salinity stress significantly reduces the plant biomass in salt sensitive wheat cultivars compared to salt tolerant (Tahir et al., 2006). Salt sensitive wheat plants showed higher decline in relative water content, membrane stability index and chlorophyll

contents with applied salt stress than salt tolerant (Sairam and Tyagi, 2004) which leads to the poor growth and biomass yield of wheat plants.

Maize (*Zea mays* L.) after wheat and rice is an important cereal crop in Pakistan. Being glycophyte, maize growth and yield retards severely as salinity increases in soil solution (Khan et al., 2006). Salinity significantly decreases the number and rate of elongation cells in maize leaves which leads to the minimal leaf growth rate (Szalai and Janda, 2009). Salt stress also reduces maize shoot growth by suppressing internode growth, as well as leaf initiation and expansion, and by accelerating leaf abscission (Qu et al., 2012). As a salt-sensitive crop, maize shoot growth is significantly reduced in initial stages of salt stress (El Sayed, 2011; Wakeel et al., 2011a)

Barley is a moderately salt tolerant crop at vegetative stage (Grewal, 2010). Different barley cultivars expressed their higher salt tolerance during growth period in such a manner as: higher stomatal conductance during the irrigation interval; growth becomes more vigorous as salinity had a little effect; higher osmotic potential inside plant body; no symptoms of salt stress on the plant height and number of productive tillers; water use efficiency shows no salinity effect but at high subsoil salinity level there was 37 and 34% decrease in shoot dry weight and water uptake of barley respectively, as compared to control (Grewal, 2010). Reduction in water uptake among barley cultivars was attributed to the root growth impediment by elevated salt concentration in soil solution (Rengasamy et al., 2003; Grewal et al., 2004a).

In pearl millet, N, P, K, Ca and Mg Concentrations were also significantly decreased with increasing NaCl concentrations, but Na and Cl accumulation increased in the plant tissues (Yakubu et al., 2010). Similarly, fresh and dry weights of shoots and roots were also decreased with NaCl application but Cl⁻ concentration increased in pearl millet tissues (Ashraf et al., 2003). Salinity stress (100 mmol L⁻¹ NaCl) significantly reduces the plant growth especially shoot length in pearl millet (Hernandez et al., 2000). The main reason behind is salt sensitive cultivar showed higher Na and Cl transfer to the shoot.

2.2.4 Salinity effects on cereals physiology

Salt stress induces osmotic effects inside plant body which alter metabolic processes and enzymatic activities. It leads to the overproduction of reactive oxygen species which causes

oxidative stress in cereals (Del Rio et al., 2006) Salt stress also enhances lipid peroxidation and antioxidative enzymes activity in roots and leaves of different maize cultivars (Neto et al., 2006). As compared to the control, guaiacol peroxidase (GPX), ascorbate peroxidase (APX), superoxide dismutase (SOD) and glutathione reductase (GR) activities are generally enhanced in salt stressed leaves of barley (Tuna et al., 2008). Salt sensitive genotypes showed less enzyme activities compared to the salt tolerant genotypes. Proline is regarded as an osmolyte and compatible solute inside plant body. It protects cereal protein against denaturation and stabilizes cell membrane by making interaction with phospholipids (Samaras et al., 1995). The proline contents were found to be increased at different osmotic potential with varying rates of salinity in the leaf tissues of various maize genotypes (Cicek and Cakirlar, 2002).

2.2.5 Reproductive stage

As physiological and developmental basis of growth and yield formation of any cereal crop in saline environment is too complex to be understood; so it is important to identify the most vulnerable aspects of growth and yield formation and focus on it (reviewed in Dolferus et al., 2011).

Pearl millet grains are rich in fat, protein content (ranges from 12-20%) and mineral elements, particularly calcium and iron (Hussain et al., 2006). These biochemical compounds were severely affected by the salinity stress. Ultimately, grain yield was significantly decreased in pearl millet with incremental rate of salinity ($EC_e = 12 \text{ dS m}^{-1}$) compared to control (Heidri and Jamshidi, 2011). There are numerous studies in cereals that have dealt with the effect of post-anthesis stress on grain-filling and grain size (Yang et al., 2006; Sinclair et al., 2006) but reduced number of grains panicle^{-1} in rice was mainly found responsible for reduction in grain yield (Mahmood et al., 2009; Dolferus et al., 2011). There was reduction in the spikelet panicle^{-1} and seed weight panicle^{-1} observed with the application of salinized water ($EC_e = 4.6 \text{ dSm}^{-1}$) at different growth stages of rice crop (Zeng et al., 2001). In rice, different yield contributing components like panicle length, 1000 grain weight, number of panicles per plant and number of tillers showed remarkable reduction when NaCl (75 mM) was applied (Abdullah et al., 2001; Shereen et al., 2005). Salinity significantly reduced the grain yield and grain quality criteria such as Beta-carotene content (ppm), gluten content (%), gluten index and protein content, of the salt sensitive wheat variety Haurani (Katerji et al., 2005). Salinity affected the durum wheat by

reducing the grain yield when the soil salinity (EC_e) was higher than 5.8 dSm^{-1} ; it is associated to the fewer grains per ear but grain yield of barley was not affected whether EC_e ranged from 0.9 to 9.8 dSm^{-1} (Katerji et al., 2009).

Grain yield was significantly reduced in a saline field when soil salinity ($EC_e < 3 \text{ dS m}^{-1}$) and irrigation water containing Na ($12 \text{ m equiv. L}^{-1}$) and Cl ($26 \text{ m equiv. L}^{-1}$) were applied in first two years. But drastic effect of soil salinity ($EC_e < 3 \text{ dS m}^{-1}$) and irrigation water Na and Cl ($34 \text{ m equiv. L}^{-1}$) on maize grain yield observed in third year. This difference in grain yield might be due to the accumulation of toxic ions in maize plant body (Isla and Aragues, 2010). About 50% yield reduction has been reported at $EC \text{ } 3.9 \text{ dS m}^{-1}$ in maize (Ayres and Westcot, 1985).

Although barley is a semi tolerant crop but similar results were also observed in barley where field screening of different cultivars carried out against soil salinity. Grain yield was significantly reduced in salt sensitive cultivars relative to salt tolerant (Isla et al., 1997). Similarly, salinity stress significantly reduced the straw yield as compared to control while remaining yield related parameters like no. of plants per m^2 , no. of ears per plant, no. of grains per year and 1000-grains weight were remained unaffected (Katerji et al., 2009).

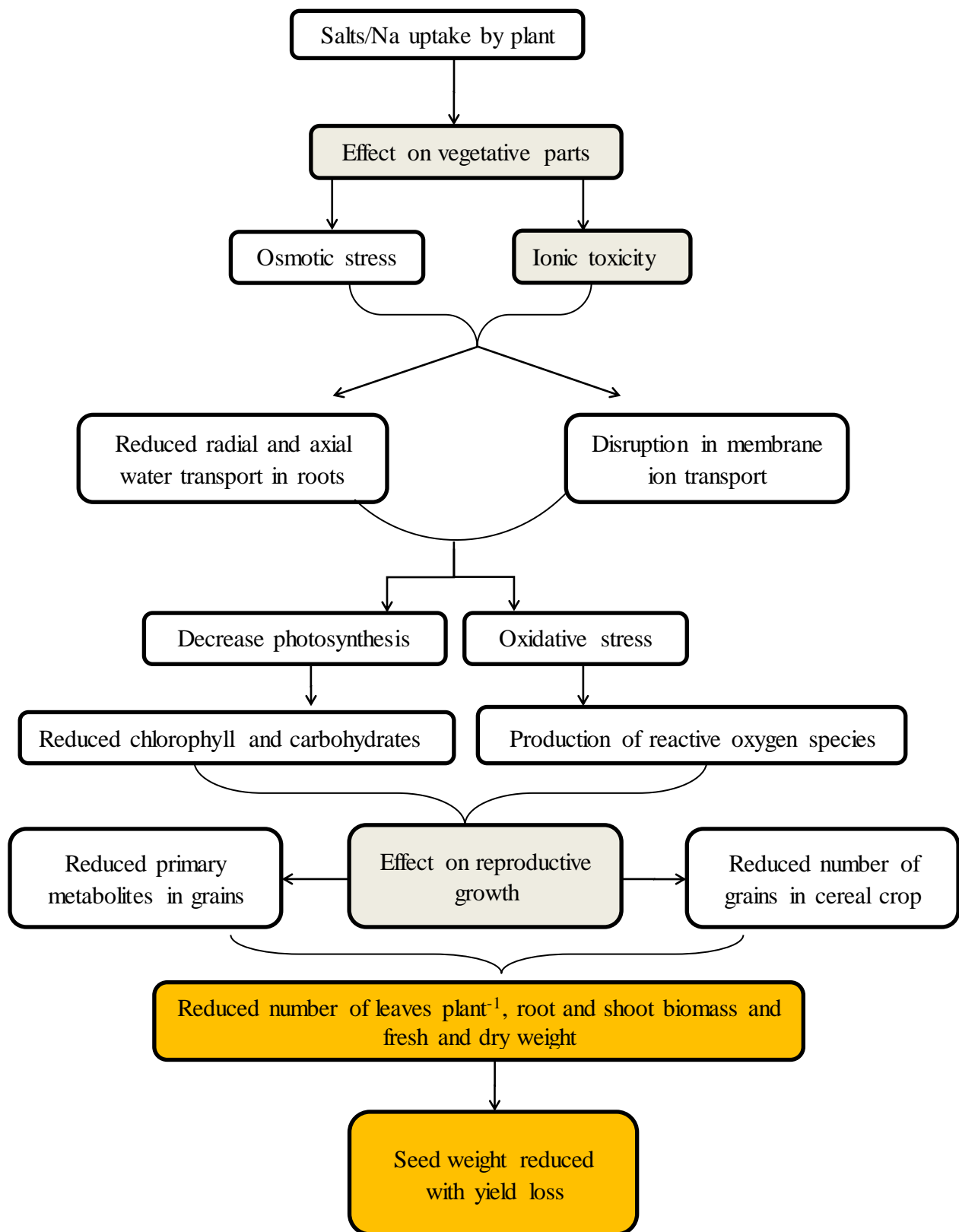


Figure2.4 Schematic diagram of salinity effect on different growth stages of cereal crops (Reviewed from Isla and Argus, 2010; Katerji et al., 2009)

2.3 Strategies to improve salinity stress

There are different strategies adopted by the cereal plants to cope up against salt stress like osmotic adjustment, avoidance of ion toxicity and nutritional balance such as increase in K^+ uptake and reduced Na^+ (Munns and Tester, 2008).

Halophytes are generally regarded as flora of salt affected soils. They are generally categorized by their potential to tolerate high Na^+ and Cl^- concentration in their shoots that can be proved lethal in glycophytes. Glycophytes are the plants that can tolerate little concentration of salt especially $NaCl \leq 200$ mM in their tissues (Flowers et al., 2015); all higher tolerant species are halophytes (Flower and Colmer, 2008). All cereals fall in the category of glycophytes but sensitivity to the salt tolerance varies from sensitive rice crop (Flowers et al., 2015) to semi tolerant barley (Tuna et al., 2008).

2.3.1 Turgor pressure

When plants grow with high concentration of salts around the root zone, they have to adjust osmotically to continue healthy growth and also maintain positive turgor pressure. Similarly, cells must maintain a total inner solute concentration higher than the external solution; but how much greater is not clearly known in cereals. The available data clearly shown that turgor pressure is generally around 0.5 MPa (Boyer, 2009). Different plant shoots vary greatly in Na^+ and Cl^- concentration; cereals generally die at the salt concentration in leaves which halophytes can tolerate. Rice cannot tolerate (on long term basis) Na^+ concentration in their leaf tissues more than 100 mM (Ul haq et al., 2013) while halophytes like *Tecticornia* spp. can maintain its growth when the tissue salt concentration is around 1.5 M Na^+ .

2.3.2 Avoidance of ionic toxicity

Sodium uptake and sequestration has always been a great interest for the researchers aimed to find the genes which can be selected to enhance salt tolerance in cereals. Different scientists also revealed that aiming Na^+ exclusion from shoots was a promising way to improve salinity tolerance in wheat (Munns et al., 2002, 2003, 2006; Munns and James, 2003; Lindsay et al., 2004). Cuin et al. (2008) compared eight wheat varieties to evaluate the root Na^+ exclusion and suggested Kharchia 65 had the highest root Na^+ exclusion ability relative to other cultivars. The plasma membrane SOS1 Na^+/H^+ antiporter were reported in transgenic Arabidopsis which

mediated the Na⁺ efflux. Similar studies were also reported in other cereal crops like sorghum (Yang et al., 1990) and maize (Fortmeier and Schubert, 1995).

2.3.3 Nutritional balance through application of mineral nutrients

Owing to continuously increasing more food and fiber demand in the world, farmers have to use the best existing technologies related to judicious application of mineral nutrition. Application of mineral nutrients is a healthy strategy and adopted by different scientists to tackle salt stress i.e. exogenous application of Ca ameliorated adverse effects of salt stress in bean (Awada et al., 1995), K in sunflower (Akram et al., 2007) and N in *Phaseolus vulgaris* (Wagenet et al., 1983). Ashraf and Foolad (2005) proposed a strategy of the exogenous application of inorganic nutrients and osmoprotectants to overcome the salt-induced injurious effect on plant growth. On the basis of this strategy, Tuna et al. (2008) have recommended the supplements of silicon (Si) to plants grown in the salt affected soils.

2.4 Silicon as a beneficial nutrient and its uptake by cereals

Silicon always remains an under-rated nutrient and its role in plant growth and physiology never got acknowledgement until the beginning of the 20th century. There are many reasons that most plant physiologists overlooked the beneficial effects of Si on plant body: First, Si remains an un-reactive element in soil plant system and secondly, it is present in the nature quite abundantly and also present as a major inorganic constituent of plants, therefore, no visible symptoms of either Si toxicity or deficiency were appeared on plants (Richmond and Sussman, 2003). There was a large amount of Si build up by certain crops especially, from Poaceae family (Mitani et al., 2005) so healthy and better growth ensured by its application to these crops. Usually higher amount of Si is deposited in the tissues of graminaceous plants relative to other species (Matichenkov and Kosobrukhov, 2004).

Silicon is classified as a quasi-essential element (Epstein, 1999). Silicic acid is the available form of Si in the soil solution (concentration upto 0.1-0.6 mM) and Si absorption takes place in monosilicic acid form by the plant roots from soil solution via transpiration stream. The polymerization of Si in the form of phytoliths (SiO₂.nH₂O) bodies takes place, when accumulation of silicic acid reaches upto a critical level of 100 mg Kg⁻¹, that comprise the bulk of a plant's Si content (Exley, 1998). In that context, significant amount of Si is present in the tissues of all

plants growing in the soil medium (Ma et al., 2001). There are two types of Si deposited layers formed within cell wall of leaves and stem; 1) silica-cuticle double layers and 2) silica-cellulose double layer (Raven, 2001). Never the less, plants accumulate it in higher amounts and it can contribute upto 0.1 to 10% of the dry matter of plants. This wide variation in Si concentration in plant tissues is attributed mainly to differences in the characteristics of Si uptake and transport (Liang et al., 2005).

Si-enriched plants are quite different from Si-deficient plants in their structure, mechanical strength, chemical composition, enzymatic activities, yield and yield contributing factors, metal toxicity, pest and disease resistance, drought and salt tolerance etc. (Epstein, 2000). Adverse effects of salinity can be minimized by the application of Si; as it plays a multitude of roles in crop performance and plant existence. Sodium uptake is reduced inside the plant when Si is present in soil solution (Tahir et al., 2012).

2.5 Distribution of silica in the mature cereal plant

Continuous deposition of silica in the plant top organs results in the increase in total silica content of cereals in all parts of the shoot with increasing age (Jones and Handreck, 1969). The significance of tissue and organ Si location was shown by the consistent increases of total plant silica, starting in the roots of cereals through the leaf sheaths to the leaf blades (Yoshido et al., 1962). There were 2.07, 12.3 and 13.4% SiO_2 observed in root, leaf sheath and blade of rice on a dry matter basis, respectively. The highest silica levels generally occur in the inflorescence bracts (Jones and Handreck, 1969). More than 90% of the total Si in plants is in form of solid silica gel in rice and the rest as soluble or colloidal forms (Sangster et al., 2001). Silica gel was present as an extracellular layer under the cuticle and compartmentalized between the cell walls and cell lumens. This cuticle-silica layer is the heaviest silica deposition site in the rice leaf and inflorescence husk (Yoshido et al., 1962). The basic silica distribution pattern was unaffected by varying the monosilicic acid content of the soil solution over the range of 7 to 67 ppm SiO_2 (Jones and Handreck, 1969). Silica content varies in wheat and barley leaves, where the younger leaves had more silica than do the mature (Sangster et al., 2001).

2.6 Silicon mediated mechanisms improving salinity tolerance

A number of economically important agronomical crops are sensitive to salt stress. Salt sensitive plants, when defined to salinity levels even low to moderate, show minimum survival. While salt-tolerant plants can survive and grow same as halophytes (Munns and Tester, 2008). Tolerance or susceptibility to abiotic stress is complex because stress can occur at various growth stages in plant life cycle (Chinnusamy et al., 2005). Different salt tolerant mechanisms in plants that can be mediated by Si are given below:

2.6.1 Formation of phytoliths

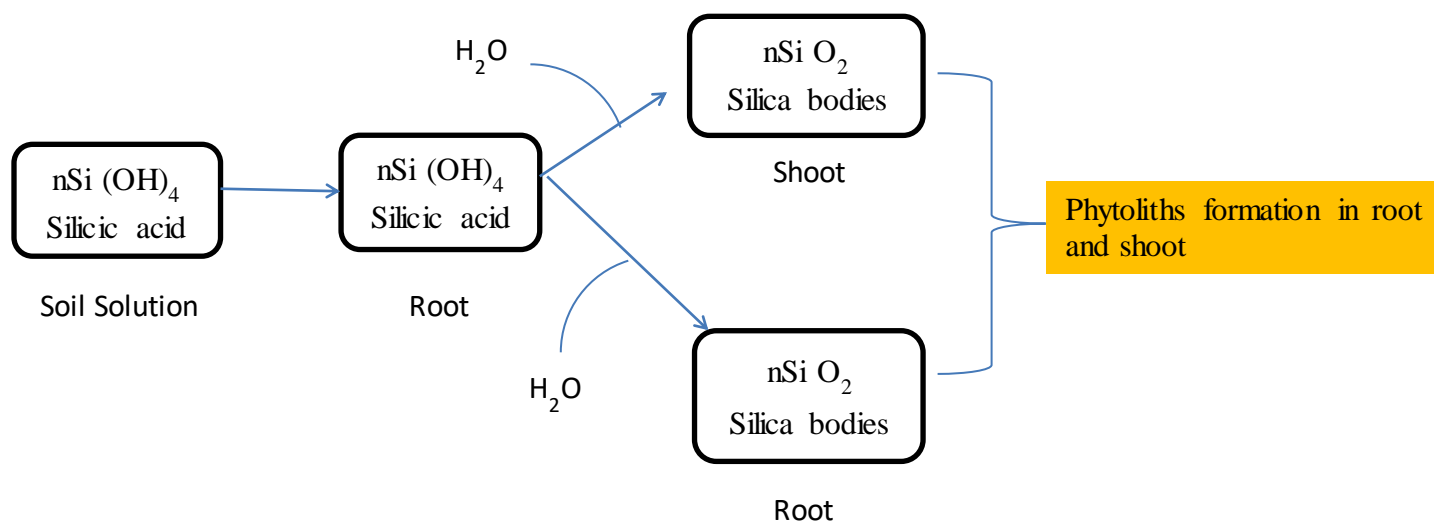


Figure 2.5 Schematic diagram of phytolith formation inside the root and shoot of cereal plants (reviewed from Cooke and Leishman, 2011)

The deposition of silica can take place anywhere in the plant body as phytoliths or discrete silica bodies (Figure 2.5) present in different shapes when they occupy the intercellular spaces. Phytoliths size and shape is determined on the basis of deposition location in the cell and size and shape of cell (Cooke and Leishman, 2011). In cereals, they adopted different shapes like dumb-bell shaped silica cells in maize (Cheng and Kim, 1989) and a leaf shaped short cell in rice (Cooke and Leishman, 2011). It is assumed that phytoliths might have the ability to bind Na⁺

with their surface so roots can enhance the uptake of K^+ from soil solution (Tahir et al., 2011). Silica deposition as phytoliths in rice and barley shoots (Yeo et al., 1999; Liang, 1996) improves the water flow through transpiration stream and reduces the translocation of Na and gives mechanical strength to the stem (Epstein, 2003). When these silica bodies present beneath the cell wall of rice and barley leaves, they not only maintain its turgor pressure but also linked with the protection of photosynthetic apparatus under salinity stress (Yeo et al., 1999; Liang, 1996).

2.6.2 Growth and Development

Silicon application to a salt stressed plant increases the shoot growth but no effect on rice root was observed by Gong et al. (2006). Silicon enhances suberization and lignification in roots of rice (Fleck et al., 2011) so radical oxygen loss in the stress condition can be minimized and plant survives under unfavorable environment. Increase in shoot dry and fresh weight and plant height has also been reported significantly by Si treatment (Shi et al., 2013). Similar findings were observed by different scientists in other crops like Si additions resulted in enhancement of dry root and shoot weight, leaf number and chlorophyll content in lettuce (Milne et al., 2012), fennel (Rahimi et al., 2012), alfalfa (Wang et al., 2011), tomato (Romero-Aranda et al., 2006) and grapevine (Soylemezoglu et al., 2009) under salinity stress. Addition of NaCl decreases the root fresh and dry weights and shoots length of maize cultivars (Parveen and Ashraf, 2010). Exogenously applied Si significantly enhances these parameters under saline regimes. Dry and fresh fennel plant weight, 1000 grain weight and grain yield enhance by Si application under salinity stress (Rahimi et al., 2012). Salinity-associated suppression was alleviated by the inclusion of 1 mM of Si in the salinized nutrient solution, so both yield and growth suppression was because of reduced net photosynthesis rate at elevated salinity level (Savvas et al., 2009). Different silicate sources were compared with sulfate sources by Abou-Baker et al. (2011) to determine the effectiveness of salt for crop. They pointed out that silicon solutions significantly increased all measured parameters as compared with sulphate solutions, although potassium silicate was the best. Potassium silicate gave the highest K% values in plant tissue in contrast to $MgSO_4$ solution which gave the highest values of N% and P%.

Silicon significantly improves wheat dry biomass when added to the salt treatment especially at the higher salt levels (100 mM NaCl) where reduction in total plant dry weight in the NaCl treatment were 39 and 54% for salt-tolerant (Izmir-85) and sensitive (Gediz-75) cultivars,

respectively (Tuna et al., 2008). Silicon addition mitigates the negative effect of Na⁺ on different growing parts of the tomato and enhances its biomass yield (Al-Aghabary et al., 2004).

2.6.3 Physiological and Biochemical

Salt stress significantly enhances H₂O₂, free proline level and malondialdehyde concentration in different crops like maize, but Si has a potential to mitigate the toxic effects of salinity on plant cellular level like root ion accumulation and proline content (Kafi and Rahimi, 2011). Application of Si improves root dry weight, root area, and leaf and root K content in the presence of salinity, but decreased leaf and root sodium (Na) content and leaf proline content in maize (Moussa, 2006). Adding Ca-silicate in salt stressed plants maintains transpiration, membrane permeability, stomatal conductance, chlorophyll content, net photosynthesis, intercellular CO₂ and reduces Na in leaves with decrease Na uptake by improving growth, balanced nutrition physiological parameters and increased nutrient uptake (Murillo-Amador et al., 2007).

Salts accumulation inside the plant body lead to the water shortage for the normal functioning of plant cells which causes physiological drought and ultimately plant cell death takes place. Major cereal crops like wheat, rice and maize are pretty responsive to the applied Si under stress conditions. Silicon was found to increase the total dry matter, relative water content, and chlorophyll content in maize cultivars (Kaya et al., 2006). Additionally, it decreases the electrolyte leakage and proline accumulation in maize plants. Silicon made salt dilution by improving the water storage within plant tissues, which allows a higher growth rate that, in turn, mitigating salt toxicity effects (Romero-Aranda et al., 2006). Silicon also significantly alleviated the NaCl adverse effects by enhancing bioactive gibberellins (GA₁ and GA₄) contents but Jasmonic acid (JA) contents sharply declined when the plants were supplemented with Si which were increased under salinity stress (Hamayun et al., 2010).

Different photosynthetic parameters like stomatal conductance, net CO₂ assimilation rate, leaf internal CO₂ concentration and transpiration rate of maize cultivars were studied by Parveen and Ashraf, (2010); they concluded that exogenously applied Si improved all those parameters both under non-saline and saline regimes. Similarly, Silicon improved photosynthetic activity by enhancing RuBisCO activity and the ultrastructure of leaf cells in barley (Liang, 1998) and reduced electrolyte leakage in the leaves enhancing the plant growth at high salinity.

Activity of antioxidant enzymes could be increased by exogenous Si application which simultaneously reduces the lipid peroxidation in roots of salt stressed barley (Liang et al., 2003). Gong et al. (2006) reported in wheat that supplementation of Si under water stress conditions increased some antioxidant enzymes activities: SOD, CAT and GR which ultimately lead to the amelioration of oxidative damage caused by ROS. Salinity stress significantly reduces the activity of SOD, CAT and APX in maize plants by enhancing the level of H₂O₂ and MDA but Si addition enhanced the SOD, CAT and APX enzymes activity (Moussa, 2006). Wang and Galletta, (1998) reported that ratios of fatty acid unsaturation enhances in phospholipids and glycolipids by foliar application of silicate in strawberry. Tuna et al. (2008) revealed that plasma membrane permeability and membrane lipid peroxidation decreases with Si application so it maintains the functions and membrane integrity of salt stressed barley, thus improving the plant growth and mitigating salt toxicity. When environmental stress was exerted on rice plants, Si improved the lipids stability in cell membranes confirming that Si prohibited the functional deterioration of cell membranes (Agarie et al., 1998). Salt stress also reduces the calcium (Ca) nutrition inside the cereal plants (Kaya et al., 2010); as Ca presence is pre-requisite for cell membrane to carry out normal functioning (Hu et al., 2007) so Si application enhances the Ca concentration inside the cereal plants especially wheat (Tuna et al., 2008). Similarly, K⁺/Na⁺ with reduced Na⁺ and enhanced K⁺ uptake and increase in wheat shoot growth was observed with Si addition under salt stress (Ali et al., 2009; Tahir et al., 2011). The possible mechanism behind reduced Na⁺ uptake is that Si deposited beneath the cell wall of roots and shoot in the form of long chains called phytoliths where these phytoliths bind Na and restrict its translocation to the upper parts of plant (Ma et al., 2003). Potassium concentration in salt stressed wheat genotypes Auqab 2000 and SARC-5 showed significant improvement when Si was applied which also reduced Na concentration by enhancing K/Na uptake (Tahir et al., 2006). Silicon also worked as a plant Na⁺ detoxification by increasing cell-wall Na⁺ binding in both salt-resistant wheat genotype SARC-1 and salt sensitive 7-Cerros (Saqib et al., 2008). Silicon application to saline medium enhances the chlorophyll content, photosynthetic activity and ribulose bis-phosphate carboxylase (RUBP) activity in leaf cell organelles and also minimizes the salt-induced H₂O₂ production (Gunes et al., 2007).

2.6 Future prospects/ Missing links

Cereals are very sensitive to grow in saline conditions. As salinity hampers their growth and minimize yield potential, so the application and accumulation of Si has increased the ability of cereal crops to maximize their growth and yield in the world where the human population is increasing and their land use activity are likely to lead increased salinization. Silicon accumulation inside plant body is almost as much as other macronutrients (Ma et al., 2001), and it is categorized as ‘quasi-essential’ element in both abiotic and biotic stress conditions. But exact mechanisms for its uptake in salt stress condition are still debatable.

There is a lot of literature available on the N, P, K solubilizing bacteria (Duponnois et al., 2006; Chuang et al., 2007) but very few studies has been reported on Si solubilizing bacteria (Sheng et al., 2008). As Si concentration is 0.1-0.6 mM in soil solution, there might be Si solubilizing bacteria involved in increasing Si concentration in soil solution which must be investigated under salt stress condition.

Similarly, there is a variety of soil microorganisms, particularly Arbuscular mycorrhizal fungi (AMF) can help plants to survive under salt stress conditions. Cereal crops showed significant improvements in physiological mechanisms when they are inoculated with AMF under stress conditions; like in maize, there is significant increase in efficiency of photosystem II, stomatal conductance, enzymatic activities (SOD, CAT) and decrease in transpiration, hydrogen peroxide and the oxidative damage to lipids (Estrada et al., 2013). All these attributes are also related to the Si concentration inside plant body. Silicon enhances the fungal growth in different nutrient solutions (Wainwright et al., 1997), so there might be fungi involved in Si translocation during its symbiotic relationship with plants, which must be explained.

Foliar application of salts is a shotgun approach to combat abiotic stress. Foliar Si application was already used to combat heavy metal toxicity like cadmium in pots (Liu et al., 2009), but no such study was yet reported on soil and foliar Si application to reduce salt toxicity in cereals under field conditions. Korndörfer et al. (2004) reported that the Si deposition under the leaf epidermis is directly related to the foliar Si application on the plants. It not only increases crop yield but also provides a physical mechanism of defense which minimizes transpiration losses, reduces lodging and enhances photosynthetic activity.

Different Si transporters have been identified in cereals like ZmLsi1 and ZmLsi6 in maize (Mitani et al., 2009) and OsLsi1, OsLsi2, and OsLsi6 from rice (Ma et al., 2006; 2007, Yamaji et al., 2008). Silicon accumulation has been attributed to the ability of the roots to take up Si (Takahashi et al., 1990), but still there is no such literature available which describes expression of the genes under salt stress condition. They must be tested under stress condition to check whether they are the main players during stress condition or some other mechanisms are being activated.

In conclusion, salinity stress generally imposes severe impacts both on human and plants by degradation of land and poor crop growth. Cereals are mostly glycophytes and higher amounts of salts in soil solution retard their growth and development irrespective of the growth stages. Early growth stages of cereal crops are most salt sensitive as compared to later growth stage. Cereal crops often cannot withstand high salinity rates until some exogenous amendment will be applied. Silicon proves to be essential in such cases under salt stress condition. It is present in higher amounts inside the plant body and prevents the crop to be transpired and lowers the activity of reactive oxygen species. It helps cereal crops to overcome stress condition in their critical growth stage and improves many physiological and biochemical mechanisms of plants. It also increases the crop biomass and yield so prove to be necessary for the cereal crop to accomplish healthy reproductive stage under salt stress. Thus, salt stress can be minimized and salt affected area must be utilized by growing cereal crops with foliar or soil Si application as an amendment. To attain this target, more field trials are required from researchers.

Chapter 3

Survey of plant tissue and soil samples for silicon concentration in different maize growing areas

Abstract

A survey was conducted in District Sargodha and Okara. The basic purpose of the survey was to estimate the soil and plant silicon (Si) concentration in the major maize growing areas of Punjab so a range of Na, K and Si concentration in maize plants were estimated to plan the future studies. Soil and plant samples were taken from adjacent areas of salt affected soils. Soils of both maize growing areas i-e District Sargodha and Okara have relatively high pH and EC. District Okara soil samples have higher EC values ($1.04\text{--}3.78\text{ dS m}^{-1}$) relative to district Sargodha ($0.72\text{--}2.54\text{ dS m}^{-1}$). There was significant positive correlation among soil and plant Si in both districts i-e Sargodha and Okara while significantly negative correlation ($r = -0.36$) found between soil Si and plant Na in District Sargodha samples. Similarly higher concentration of soil Si was found in district Okara samples (0.57 mg Kg^{-1}) relative to district Sargodha (0.47 mg Kg^{-1}) which leads to the higher plant Si concentration. District Okara plant samples have higher Na/K ratio relative to District Sargodha. Farmers generally do not grow maize on salt affected soils in these areas, so we cannot get any toxic effects of Na or deficiency (Si) in our samples.

3.1 Introduction

Food security is a serious issue for developing countries because of poor resources, high population pressure and lack of management both in food production and its distribution. Population of Pakistan is also increasing at a tremendous rate and there is dire need to improve crop yields to meet the food demands. Two basic approaches generally discussed for increased food production are i) to increase area under production and ii) to increase yield per hectare. To increase area under production is nearly impossible for a number of reasons; rather it is being decreased because of increased urbanization, industrialization and desertification.

Soil salinity is a serious agricultural and environmental problem affecting crop production (Ashraf et al., 2008; Ashraf, 2009) around the globe and is a big cause of desertification. There is dire need to reclaim salt affected soils to sustain agricultural productivity and to increase area under crop production. Exact survey of the area for soil quality parameters is pre-requisite for any management strategies meant for reclaiming the degraded soils.

Silicon application can moderate the salinity stress in plants and plays a multitude of roles in plant existence and crop performance (Tahir et al., 2012). Silicon application reduces Na^+ uptake (Tahir et al., 2011) by making complex with Na^+ in soil (Ahmad et al., 1992). Silicon is deposited in leaves resulting decreased transpiration and hence dilutes salts accumulation (Matoh et al., 1986). Gramineous plants accumulate more Si in their tissues than other species (Matichenkov and Kosobrukhov, 2004).

Maize grain is a rich source of starch, vitamins, proteins and minerals, gives the highest conversion of dry substance to meat, milk and eggs compared to other cereal grains. Being glycophytic plant, maize is severely affected by salinity. Survey to know the extent of problem/salinity is pre-requisite for a proper management plan as variation in salinity exists in soils. Moreover, there is no reference about the Si status in soils of Pakistan. Hence, a survey of maize growing areas for soil salinity and Si contents was carried out.

3.2 Materials and methods

The survey was carried out in the selected maize growing zone (Sargodha, Bhalwal, Okara and Depalpur) of Punjab, Pakistan by sampling maize leaves (adjacent to the flag leaf) and associated soils within about 5 m². The total 50 sites were surveyed during 2013. X and Y coordinates of each sampling sites were recorded using Garmin e Trex Hiking global positioning system (GPS). Soil and leaf samples thus collected were brought to the laboratory. Soil samples were dried, ground and sieved. A sub sample of the sieved soil was analyzed for pH, electricity conductivity, extractable sodium and potassium (Richards, 1954) and extractable Si (Elliot and Synder, 1991). For analysis, leaf samples were washed with distilled water and ground with a Wiley mill fitted with a stainless steel chamber and blades. Samples were prepared and analyzed in triplicates.

Finely ground plant samples (0.1 g) were digested in a di-acid ($\text{HNO}_3\text{:HClO}_4$) mixture (Jones and Case, 1990). The Na and K concentration in the digest was estimated by flame photometer

(Jenway, PFP-7). For Si determination, the ground samples (0.2 g) were digested in 2 mL 50% hydrogen peroxide (H_2O_2) and 4.5 mL 50% NaOH in open vessels (Teflon beakers) on a hot plate at 150°C for 4 hours. Silicon concentration was measured using calorimetric amino molybdate blue color method (Elliot and Synder, 1991) on UV-visible spectrophotometer (Shimadzu UV-1201). To 1 mL of supernatant filtrate liquid, 10 mL of ammonium molybdate (54 g L^{-1}) solution and 25 mL of 20% acetic acid was added in 50 mL of polypropylene volumetric flask.

After five minutes, 5 mL of 20% tartaric acid and 1 mL of reducing solution was added in flask and volume was made with 20% citric acid. After 5 but not more than 30 minutes, the absorbance was measured at 650 nm wave length with a UV visible spectrophotometer (Shimadzu, Spectronic 100, Japan). The reducing solution was made by combining solution A (2 g of Na_2SO_3 in 25 mL of DM plus 0.5 g of l-amino-2-naphthol-4-sulfonic acid) and solution B (25 g of NaHSO_3 dissolved in about 200 mL of DM) and diluting to 250 mL.

Statistical analysis

Data from the whole experiment were tabulated in Microsoft Office Excel 2007. Software package statistic v. 8.1 was used for the calculation of standard error and correlation.

3.3 Results

3.3.1 Sargodha District

3.3.1.1 Soil pH

All of the samples were alkaline and pH of surface soil (0-15 cm) ranged from 7.75-8.40 with mean value of 8.00 (SD = 0.16) (Table 3.1a). Maximum soil pH (8.40) was observed at Nawab Chak (site no. 6) while minimum value was 7.75 at Ludhay Wala and Uttian Sharif (site no. 7 and 18). The data indicated that majority of the area was alkaline in reaction because of high pH values ($\text{pH} > 7.0$) and about 50% samples had $\text{pH} > 8.00$.

3.3.1.2 Electrical conductivity of a soil saturation extract (EC_e)

The EC_e of soil depth (0-15 cm) ranged from 0.72-2.54 with mean value of 1.43 dS m^{-1} (SD = 0.47) (Table 1). Maximum soil EC_e in Sargodha district (2.54 dS m^{-1}) was observed at site no. 31(Chak 22, Farm House) while minimum value was 0.72 dS m^{-1} at site no. 01 (Rajua Sadat).

It was clear from surface soil result (Table 3.1) that out of 31 samples of Sargodha District, only three samples had $EC > 2 \text{ dS m}^{-1}$ while remaining samples had EC_e value $< 2 \text{ dS m}^{-1}$.

3.3.1.3 Silicon concentration in soil and of maize plants

The soil Si concentration in Sargodha district ranged from $0.232\text{-}0.788 \text{ mg g}^{-1}$ with mean concentration of 0.47 mg g^{-1} (SD= 0.164). The maximum concentration of extractable soil Si 0.788 mg g^{-1} was recorded at Chak 17 shumali while it was minimum 0.232 mg g^{-1} at Peelawal (Table 3.1).

Tehsil Bhalwal shows 115 and 128 % higher in soil Si concentration when compared to its adjacent areas, Ajnala Lok and Chak 29 shumali, respectively. Similarly, Tehsil Maari has 108 and 13% higher in soil Si concentration when compared to its adjacent areas, Chak 54 shumali and chak 55 shumali, respectively. There was two folds higher soil Si concentration in Chak 33 shumali when compared to Chak 29 shumali.

3.3.1.4 Silicon concentration in leaves of maize plants

The maize leaf Si concentration in Sargodha district ranges from 0.574 to 1.60 % with mean concentration of 1.00 % (SD= 0.03). The maximum concentration of leaf Si 1.60 % was recorded at Chak 66 while it was minimum 0.57 % at Rajua sadat (table 3.1). Tehsil Bhalwal shows 43 and 124% increase in leaf Si concentration when compared to Chak 29 shumali and Purana Bhalwal.

3.3.1.5 Sodium concentration in soil of maize plant

In the different regions of Sargodha district, extractable soil Na concentration varies from 66-177 mg kg^{-1} with mean concentration of 115 mg kg^{-1} (SD=0.58). Tehsil Bhalwal had the maximum soil Na concentration (177 mg kg^{-1}) while Chak 22 showed the minimum soil Na concentration (66 mg kg^{-1}). Different adjacent areas showed remarkable differences in soil Na

concentration. Chak 40 N-B produced 45 and 59 % higher soil Na concentration as compared to Chak 52 shumali and teen pullian, respectively. Similarly, Purana Bhalwal had 63 and 52% more soil Na concentration when compared to Chak no.10 and Ajnala, respectively. Thus, it implies that Tehsil Bhalwal had maximum extractable soil Na concentration in District Sargodha which ultimately affected the soil structure and its physic-chemical properties.

3.3.1.6 Sodium concentration in leaves of maize plant

There was a different pattern observed in leaves Na concentration as compared to soil Na concentration. Leaves Na concentration ranged from 4.0-8.1 mg g⁻¹ with the mean concentration of 5.8 mg g⁻¹ (SD=0.143). Sodium concentration varied significantly among different tehsils of Sargodha district (Table 3.1). Uttian sharif showed 47 and 51% higher Na concentration in maize leaves when compared to Bhalwal and Maari. Ajnala lok had 26% higher Na concentration in maize leaves as compared to Purana Bhalwal. It might be due to genotypic variation in Tehsil Bhalwal that either the cultivars do not uptake the Na⁺ and avoid the specific ion toxicity or they accumulate the Na⁺ into their vacuole and ultimately growth remains unaffected in salt stressed conditions.

3.3.1.7 Potassium concentration in soil of maize plant

Out of 31 soil samples of Sargodha district, 20 samples showed more than 100 mg kg⁻¹ extractable K concentration which clearly indicated that adequate amount of extractable K is available in the soil to combat Na toxicity. Generally, K concentration in Sargodha soils ranged from 68.8 mg kg⁻¹ (Chak 22 Farm house) to 190 mg kg⁻¹ (Chak 33 shumali) with mean value 107 mg kg⁻¹ (SD= 0.4). There was a significant increase in the soil K concentration in Chak no. 29 shumali (162 mg kg⁻¹) which is 54 and 66% higher than its adjacent areas i-e; Ajnala Lok and Bhalwal, respectively.

3.3.1.8 Potassium concentration in leaves of maize plant

Potassium concentration in maize leaves varied from 2.3-7.0 mg g⁻¹ with the mean value 4.2 mg g⁻¹. Maximum K concentration (7.0 mg g⁻¹) in leaves was observed in the plant samples of Chak 53 shumali while the least (2.3 mg g⁻¹) was in Chak 55 shumali. Suleman Pura and Purana Bhalwal had 45.5% higher K concentration in maize leaves then Tehsil Bhalwal. Uttian sharif

also provides two times higher K concentration in maize leaves than Chak 40 N-B. Thus, it implies from the data that some tehsils and villages i.e. Rajua Saadat, Chak 33 shumali, Chak no. 29 shumali have higher K concentration in maize leaves than others i.e. Bhalwal, Ajnala lok and Chak 40N-B, so they have better potential to sustain under salinity stress.

3.3.1.9 Na/K ratio in maize leaves

Mean Na/K ratio in leaves of maize was 1.49 and ranged from 0.87-3.24 (Table. 3.1). In leaves, cell membrane integrity and selectivity is disrupted under saline conditions due to high levels of Na^+ in soil solution that also interferes with K^+ acquisition by the cells.

3.3.1.10 Relation (Pearson correlation coefficient, r) between Si and soil physio-chemical properties (0-15 cm depth)

Correlation analysis was made for 5 soil properties representing soil physico-chemical and chemical/nutrient which indicated intra and inter-relationships among the soil properties. Of 10 pairs in correlation matrix, 1 pair (soil Si and Na) showed significant negative relationship ($r = -0.36$) (Table 3.2).

Table 3.1 Soil and plant parameters of district Sargodha including different concentrations of Si, K and Na

| Locations | Site no. | <u>Soil parameters</u> | | | | | <u>Plant parameters</u> | | | |
|--------------------|--------------|------------------------|-----------------------------|------------------------------|------------------------------|-----------------------------|-------------------------|-----------------------------|----------------------------|-----------|
| | | pH | EC (dS m ⁻¹) | Si (mg kg ⁻¹) | Na (mg kg ⁻¹) | K (mg kg ⁻¹) | Si (%) | Na (mg g ⁻¹) | K (mg g ⁻¹) | Na/K |
| Rajua Saadat | 1 | 8.11 | 0.722 | 0.301 | 126 | 111.28 | 0.574 | 6.8 | 5.2 | 1.31 |
| Peelowal | 2 | 8.23 | 1.028 | 0.232 | 137 | 103.02 | 0.707 | 7.0 | 3.5 | 2.00 |
| Chak 40 N-B | 3 | 8.1 | 0.837 | 0.493 | 147 | 74.12 | 0.770 | 7.3 | 2.8 | 2.58 |
| Chak 52 shumali | 4 | 8.05 | 0.897 | 0.346 | 101 | 133.70 | 0.663 | 4.9 | 4.0 | 1.20 |
| Teen pulan | 5 | 8.02 | 1.363 | 0.625 | 92 | 103.02 | 1.157 | 4.0 | 4.6 | 0.88 |
| Nawab chak | 6 | 8.4 | 1.634 | 0.549 | 110 | 131.34 | 1.278 | 6.5 | 3.2 | 2.01 |
| Ludhay wala | 7 | 7.75 | 1.652 | 0.435 | 122 | 140.77 | 1.234 | 5.6 | 4.7 | 1.19 |
| Chak 66 | 8 | 7.87 | 1.615 | 0.562 | 107 | 111.28 | 1.602 | 7.3 | 4.0 | 1.79 |
| Chak 55 shumali | 9 | 7.88 | 1.531 | 0.460 | 102 | 73.53 | 0.770 | 7.6 | 2.3 | 3.24 |
| Chak 55 shumali | 10 | 7.85 | 1.571 | 0.372 | 91 | 117.77 | 0.751 | 4.8 | 4.8 | 1.00 |
| Chak 55 shumali | 11 | 7.77 | 0.743 | 0.745 | 93 | 80.01 | 0.834 | 5.0 | 4.4 | 1.13 |
| Chak 55 shumali | 12 | 8.06 | 2.135 | 0.488 | 94 | 52.29 | 1.284 | 5.7 | 3.9 | 1.45 |
| Chak 54 shumali | 13 | 8.09 | 1.754 | 0.273 | 111 | 51.70 | 1.386 | 5.7 | 4.8 | 1.19 |
| Chak 54 shumali | 14 | 7.9 | 1.683 | 0.232 | 103 | 117.18 | 0.726 | 7.8 | 4.2 | 1.86 |
| Maari | 15 | 8.21 | 1.544 | 0.499 | 130 | 122.49 | 1.221 | 4.0 | 3.8 | 1.04 |
| Maari | 16 | 8.04 | 1.674 | 0.554 | 137 | 144.31 | 0.929 | 4.5 | 5.2 | 0.87 |
| Uttian sharif | 17 | 7.89 | 0.941 | 0.628 | 123 | 134.87 | 1.386 | 8.1 | 5.6 | 1.45 |
| Uttian sharif | 18 | 7.75 | 0.825 | 0.486 | 110 | 103.61 | 1.088 | 7.7 | 6.2 | 1.24 |
| Chak 17 shumali | 19 | 7.81 | 0.871 | 0.260 | 91 | 110.69 | 0.713 | 5.5 | 4.6 | 1.18 |
| Chak 17 shumali | 20 | 7.96 | 1.003 | 0.788 | 102 | 72.35 | 0.967 | 3.6 | 3.5 | 1.04 |
| 18 waali nehr | 21 | 8.06 | 1.908 | 0.316 | 113 | 126.62 | 0.599 | 5.6 | 3.3 | 1.67 |
| suleman pura | 22 | 8.3 | 1.676 | 0.372 | 137 | 151.39 | 0.777 | 6.6 | 4.8 | 1.39 |
| Purana Bhalwal | 23 | 8.27 | 2.104 | 0.554 | 141 | 134.87 | 0.485 | 5.7 | 4.8 | 1.18 |
| Chak 10 | 24 | 8.09 | 1.997 | 0.493 | 86 | 113.64 | 0.929 | 5.4 | 5.6 | 0.96 |
| Ajnala | 25 | 7.94 | 1.566 | 0.511 | 96 | 75.88 | 0.961 | 5.9 | 4.2 | 1.40 |
| Bhalwal | 26 | 7.93 | 1.635 | 0.554 | 177 | 68.81 | 1.088 | 5.5 | 3.3 | 1.69 |
| Ajnala Lok | 27 | 8.01 | 1.117 | 0.257 | 155 | 75.88 | 1.094 | 7.6 | 3.2 | 2.35 |
| Chak 29 shumali | 28 | 7.87 | 1.709 | 0.235 | 168 | 162.01 | 0.751 | 7.2 | 6.8 | 1.07 |
| Chak 33 shumali | 29 | 7.8 | 1.223 | 0.460 | 126 | 190.33 | 1.449 | 6.4 | 7.0 | 0.91 |
| Chak 22 Farm house | 30 | 8.11 | 0.855 | 0.752 | 78 | 72.35 | 1.234 | 2.5 | 2.6 | 0.95 |
| Chak 22 Farm house | 31 | 7.93 | 2.54 | 0.757 | 66 | 68.81 | 1.595 | 4.0 | 3.5 | 2.91 |
| | Range | 7.75-8.4 | 0.72-2.54 | 0.232-0.788 | 66-177 | 68.8-190.3 | 0.574-1.602 | 4.0-8.1 | 2.3-7.0 | 0.87-3.24 |
| | mean | 8.00 | 1.43 | 0.47 | 115 | 107.3 | 1.000 | 5.8 | 4.2 | 1.49 |
| | SD | 0.17 | 0.47 | 0.164 | 0.58 | 0.40 | 0.030 | 0.143 | 0.126 | 0.61 |

Table 3.2 Relationship among various parameters of the tested soil samples (0-15 cm depth) of Sargodha district. Total number of soil samples was 31. Each sample was taken from three sites and mixed them to form one composite sample.

| | EC | K | Na | Si |
|-----------|-----------|----------|-----------|-----------|
| K | 0.02 | | | |
| Na | 0.07 | 0.30 | | |
| Si | -0.19 | -0.27 | -0.36* | |
| pH | 0.12 | 0.02 | 0.18 | -0.06 |

*significant (P<0.05)

3.3.1.11 Relationship (Pearson correlation coefficient, r) of leaf composition parameters with soil properties (0-15 cm depth)

Of 20 pairs in correlation matrix, 2 pairs showed highly significant relationships among them, while 3 pairs showed significant relationships among them (Table 3.3). Highly significant positive relationship was observed between soil K and plant K ($r = 0.64$); and highly significant negative relationship was observed between soil Si and plant Na ($r = -0.53$), respectively (Table 3.3b). There was also significant positive relationship found between soil Si and plant Si.

Table 3.3 Relationship (Pearson correlation coefficient, r) of maize plant parameters with soil properties (0–15 cm depth) of Sargodha district. Total number of samples was 31.

| Plant Parameters Soil Parameters | Na | K | Na:K | Plant available Si |
|---|-----------|----------|-------------|---------------------------|
| EC_e | 0.13 | 0.14 | -0.05 | -0.25 |
| pH | -0.11 | -0.26 | 0.06 | -0.17 |
| Na | 0.45* | 0.24 | 0.04 | -0.21 |
| K | 0.20 | 0.64** | -0.39* | -0.16 |
| Si | -0.53** | -0.29 | -0.05 | 0.43* |

**highly significant (P<0.01), *significant (P<0.05)

3.3.2 Okara District

3.3.2.1 Soil pH

All of the soil samples from Okara were alkaline and pH of surface soil (0-15 cm) ranged from 7.4-8.30 with mean value of 7.79 (SD = 0.29) (Table 3.2). Maximum soil pH 8.30 was observed at University Farm at Haveli lakha (site no.12) while minimum value was 7.40 at Bunga Hayat (site no. 18). The data indicated that all of the areas were alkaline in nature because of high pH values (pH > 7.0). So, overall all data indicated that 25% samples had pH > 8.00, which ultimately caused high Na and low K availability in soil.

3.3.2.2 Electrical conductivity of a soil saturation extract (EC_e)

The EC_e of soil depth (0-15 cm) ranges from 1.04-3.78 with mean value of 1.84 dS m⁻¹ (SD = 0.74) (Table 2). Maximum soil EC_e in Okara district (3.78 dS m⁻¹) was observed at Chura manika (site no. 11) while minimum value was 1.0 dS m⁻¹ at Wasaway wala (site no. 06).

3.3.2.3 Silicon concentration in soil of maize plant

The concentration of extractable surface soil Si (0-15 cm) ranged from 0.387-0.971 mg g⁻¹ with mean concentration of 0.571 mg g⁻¹. The maximum concentration of extractable Si 0.971 mg g⁻¹ was recorded at Mehtab Garh (site no. 16) while it was minimum 0.387 mg g⁻¹ at University Farms, Havelilakha (site no. 13).

3.3.2.4 Silicon concentration in leaves of maize plant

The leaf Si concentration in Okara district ranged from 0.472 - 1.399 % with mean concentration of 1.026 % (SD= 0.291). The maximum concentration of leaf Si 1.399 % was recorded (Table 3.2) at Anok Singh (site no.8) while it was minimum 0.472 % at University Farms, Havelilakha (site no. 15).

3.3.2.5 Sodium concentration in soil of maize plant

In the different regions of Okara district, Na concentration varied from 63- 148 mg kg⁻¹ with mean concentration of 91.09 mg kg⁻¹ (SD=0.27). University Farm at Havelilakha had the maximum soil Na concentration (148 mg kg⁻¹) while Bunga hayat showed the minimum soil Na concentration (63 mg kg⁻¹). Different adjacent areas showed remarkable differences in soil Na

concentration. University Farm at Havelilakha (site no. 15) has 48, 59 and 41 % higher soil Na concentration as compared to Mehtab garh, Bunga hayat and Sukh pur, respectively. Similarly, soil Na concentration increases 11 and 17% in Dola pukhta soil samples when compared to Wasaway wala and Anok Singh, respectively.

3.3.2.6 Sodium concentration in leaves of maize plant

There was much higher leaf Na concentration observed in Okara maize plant samples as compared to Sargodha District leaf samples (Table 3.2). Leaf Na concentration ranged from 3.7-9.5 mg g⁻¹ with the mean concentration value of 6.9 mg g⁻¹ (SD=0.206). Sodium concentration varied significantly among different villages and Tehsil havelilakha of Okara district (Table 3.2). University Farm at Havelilakha had 60 and 101% higher Na concentration in maize leaves when compared to Churamanika and Kali peeran wali. It is concluded that higher Na⁺ concentration in the soil and maize crop is the major reason of poor cereal growth in district Okara especially in tehsil Havelilakha.

3.3.2.7 Potassium concentration in soil of maize plant

Extractable soil potassium concentration in District Okara ranged from 51.1 mg kg⁻¹ (Jalal Kot Chak 15-D) to 113.6 mg kg⁻¹ (Churamanika) with mean value of 78.67 (SD= 0.283). There was a two fold increase in the soil extractable K concentration in Kali peeran wali (105.38 mg kg⁻¹) and Churamaninka (113.38 mg kg⁻¹) as compared to its adjacent areas i-e; Jalal Kot Chak 15-D (51.11 mg kg⁻¹) and Farm at Havelilakha (55.38 mg kg⁻¹), respectively. Out of 19 soil samples of Okara district, only 5 samples showed more than 100 mg kg⁻¹ extractable soil K concentration which is clearly indicated that adequate amount of extractable K is not available in the soil to combat Na toxicity.

3.3.2.8 Potassium concentration in soil of maize plant

Potassium concentration in maize plant leaves varies from 2.6-5.3 mg g⁻¹ with the mean value 3.74 mg g⁻¹ (SD=0.84). Maximum K concentration (5.3 mg g⁻¹) in leaves was observed in the plant samples of Mehtab garh while the least (2.6 mg g⁻¹) was at University Farm at Havelilakha. The trend of K concentration in most of the maize leaf analysis (15 samples) generally remains constant in between 3.0-4.5 mg g⁻¹. Potassium concentrations was higher 65.6 and 78.4% in

Mehtab garh maize leaf samples relative to Tehsil Bunga hayat and university Farm at Havelilakha. Thus, it implies from the data that few villages like Mehtab garh and Wasaway wala have higher K concentration in maize leaves then others, so they have batter potential to sustain under salt stress.

3.3.2.9 Na/K ratio in maize leaves

Mean Na/K ratio in leaves of maize was 1.31 and ranged from 0.62-2.99 (Table. 3.4). In leaves, cell membrane integrity and selectivity is disrupted under saline conditions due to high levels of Na^+ in soil solution that also interferes with K^+ acquisition by the cells.

Table 3.4 Soil and plant parameters of District Okara including different concentrations of Na, K and Si

| Locations | Site no. | <u>Soil parameters</u> | | | | | <u>Plant parameters</u> | | | |
|-----------------------------------|----------|----------------------------|-----------|------------------------------|------------------------------|-----------------------------|-----------------------------|----------------------------|-----------|-----------------|
| | | pH (dSm ⁻¹) | EC | Si (mg kg ⁻¹) | Na (mg kg ⁻¹) | K (mg kg ⁻¹) | Na (mg g ⁻¹) | K (mg g ⁻¹) | Na/K | Si (%) |
| Deepal pur road Okara | 1 | 7.6 | 1.837 | 0.483 | 66 | 106.56 | 4.9 | 3.2 | 1.51 | 1.234 |
| Chak 44/D | 2 | 7.95 | 1.041 | 0.448 | 77 | 74.71 | 4.0 | 3.5 | 1.12 | 0.967 |
| Haweli lakha road | 3 | 7.89 | 1.704 | 0.410 | 94 | 69.99 | 6.0 | 3.2 | 1.91 | 1.386 |
| Dola pukhta | 4 | 7.7 | 1.326 | 0.280 | 102 | 63.50 | 5.2 | 4.6 | 1.12 | 0.580 |
| Jalal kot chak 15D | 5 | 8.19 | 1.741 | 0.514 | 91 | 51.11 | 4.3 | 4.2 | 1.03 | 0.904 |
| Wasaway wala | 6 | 7.74 | 1.009 | 0.641 | 87 | 66.45 | 4.5 | 5.2 | 0.88 | 1.234 |
| Anok singh | 7 | 7.34 | 2.05 | 0.702 | 82 | 72.94 | 5.7 | 3.2 | 1.79 | 1.157 |
| Anok singh | 8 | 7.95 | 2.53 | 0.689 | 94 | 78.24 | 7.8 | 3.3 | 2.36 | 1.399 |
| Anok singh | 9 | 7.84 | 1.788 | 0.410 | 83 | 69.99 | 3.7 | 3.4 | 1.08 | 0.777 |
| kali peran wali, haweli lakha | 10 | 8.1 | 1.166 | 0.493 | 102 | 105.38 | 6.3 | 4.8 | 1.33 | 0.967 |
| Chura manika | 11 | 8.01 | 3.78 | 0.499 | 82 | 113.64 | 4.1 | 4.5 | 0.91 | 0.789 |
| University Farm at Havelilakha | 12 | 8.3 | 1.145 | 0.483 | 108 | 52.29 | 5.1 | 2.6 | 1.95 | 1.234 |
| University Farm at Havelilakha | 13 | 7.99 | 2.02 | 0.387 | 114 | 71.17 | 8.2 | 3.0 | 2.75 | 0.751 |
| University Farm at Havelilakha | 14 | 7.5 | 2.2 | 0.691 | 148 | 55.83 | 9.0 | 3.0 | 2.96 | 0.650 |
| University Farm at Havelilakha | 15 | 8 | 1.262 | 0.554 | 132 | 44.03 | 9.5 | 2.6 | 3.68 | 0.472 |
| Mehtab garh | 16 | 7.51 | 1.198 | 0.971 | 70 | 107.74 | 2.6 | 5.3 | 0.48 | 1.221 |
| Bunga hayat | 17 | 7.51 | 2.56 | 0.894 | 64 | 73.53 | 2.0 | 3.2 | 0.62 | 1.386 |
| Bunga hayat | 18 | 7.4 | 1.58 | 0.747 | 63 | 113.64 | 4.0 | 3.9 | 1.04 | 1.297 |
| Sukh Pur | 19 | 7.41 | 3.06 | 0.562 | 73 | 104.20 | 4.0 | 4.5 | 0.87 | 1.094 |
| | Range | 7.4-8.3 | 1.04-3.78 | 0.387- 0.971 | 63-148 | 51.1- 113.6 | 3.7-9.5 | 2.6-5.3 | 0.48-3.68 | 0.472- 1.399 |
| | Mean | 7.79 | 1.84 | 0.571 | 91.09 | 78.67 | 6.9 | 3.74 | 1.55 | 1.026 |
| | SD | 0.29 | 0.74 | 0.176 | 0.27 | 0.283 | 0.206 | 0.85 | 0.86 | 0.291 |

3.3.2.10 Relation (Pearson correlation coefficient, r) between Si and soil physio-chemical properties (0-15 cm depth)

The r value for soil extractable Si with Na, K and EC_e, pH were obtained -0.27, 0.24 and 0.10, -0.53, respectively (Table 3.5). Soil Si only showed significantly negative correlation with soil pH (r= -0.53). Similarly, Soil Na also showed highly significant negative correlation with soil K (r= -0.63).

Table 3.5 Relationship among various parameters of the tested soil samples (0-15 cm depth) of Okara district. Total number of soil samples was 19. Each sample was taken from three sites in one field and mixed them to form one composite sample.

| | K | Na | Si | EC |
|----|---------|-------|--------|-------|
| Na | -0.64** | | | |
| Si | 0.24 | -0.28 | | |
| EC | 0.34 | -0.16 | 0.10 | |
| pH | -0.37 | 0.37 | -0.53* | -0.21 |

**highly significant (P<0.01), *significant (P<0.05)

3.3.2.11 Relationship (Pearson correlation coefficient, r) of leaf composition parameters with soil properties (0-15 cm depth)

There was significant negative correlation was found between Na concentration of the leaves with measured soil K, but leaf Na showed highly significant positive correlations to the soil extractable Na concentration and Na/K ratio (Table 3.6). Of 20 pairs in correlation matrix, 2 pairs showed highly significant relationships among them, while 3 pairs showed significant relationships (Table 3.6). Highly significant positive correlation includes (r= 0.49) plant Na/K ratio to the soil extractable Na concentration; while other highly significant positive correlation was observed between plant Na and soil Na (r= 0.85). There was also significant positive relationship found between soil Si and plant Si (r= 0.46). Leaf K concentration was also significant positively

correlated with soil K ($r = 0.49$) but significant negatively correlated soil Na/ K ratio ($r = -0.48$). However, most of the soil properties were negatively correlated with each other's.

Table 3.6 Relationship (Pearson correlation coefficient, r) of maize plant parameters with soil properties (0–15 cm depth) of Okara district. Total number of samples was 19.

| <div>Plant Parameters</div> <div>Soil Parameters</div> | Na | K | Na:K | Plant available Si |
|--|--------|-------|--------|--------------------|
| EC _e | -0.06 | -0.04 | -0.06 | 0.02 |
| pH | 0.26 | -0.16 | 0.25 | -0.23 |
| Na | 0.86** | -0.37 | 0.82** | - 0.20 |
| K | -0.43* | 0.49* | -0.53 | 0.35 |
| Si | -0.26 | 0.16 | -0.20 | 0.46* |

**highly significant ($P < 0.01$), *significant ($P < 0.05$)

3.4 Discussion

It was clear from surface soil result of Okara district (Table 3.4) that out of 19 samples, only two samples had $EC > 3 \text{ dS m}^{-1}$ while remaining samples had EC_e value $< 2 \text{ dS m}^{-1}$. The higher soil salinity values at the surface of the soil might be due to accumulation of salts (Ca^{+2} , Mg^{+2} , CO_3 , HCO_3^{-1} , SO_4 , Cl^{-1}) in upper layer which were accumulated due to the water evaporation from soil surface and low rain fall (arid to semi-arid climate). Hussain et al. (2006) reported that application of brackish water from drains having high EC, SAR and RSC to grow crops due to shortage of good quality water in addition to salty parent material is the reason of increasing salt balance in soils.

Relatively high pH of these soils (Table 3.1, 3.4) might be due to medium to high base saturation of soils (Kumar et al., 1997). Soil EC_e values decreased with depth. Application of brackish water from drains having high EC, SAR and RSC to grow crops due to shortage of good quality

water in addition to salty parent material is the reason of increasing salt balance in soils (Hussain et al., 2006). Under saline conditions, elevated Na^+ concentration hampers the growth and development of plants as it suppress the photo-system II photochemical activity and leaf gas exchange (Dionisio-Sese and Tobita, 2000b). The major reason of reduced growth of cereal crops in salt stressed condition is specific ion toxicity (certain ions like Na and Cl uptake at elevated level) (Chinnusamy et al., 2005; Tahir et al., 2012). There is considerable evidence that Na exclusion is the mechanism for toxicity avoidance of the most important crops such as maize (Fortmeier and Schubert, 1995). Potassium is the only monovalent cation that is essential for mostly higher plants and is involved in three important functions, i.e., enzyme activation, charge balance, and osmoregulation in plants (Mengel, 2007). A number of studies in many crops have shown that K^+ concentration in plant tissues, expressed on dry matter basis, reduces as the $\text{Na}^+/\text{Ca}^{2+}$ in the root media increases (Hu and Schmidhalter, 2005). High level of external Na^+ caused a decrease in both K^+ and Ca^{2+} concentrations in the plant tissues of many plant species (Hu and Schmidhalter, 2005); there was significant negative correlation found between soil Na and plant K in Okara district samples (Table 3.6).

Silicon application can moderate the salinity stress in plants and plays a multitude of roles in plant existence and crop performance. Silicon application reduces Na^+ uptake by making complex with Na^+ in soil (Ahmad et al., 1992). Silicon is deposited within cell wall forming silica-cuticle double layers and silica-cellulose double layer on the surfaces of leaves and stem (Raven, 1983). About 55 % of leaf samples had a Si concentration below 1% (Table 3.1) and are regarded as Si deficient plants in abiotic stress condition (Liang et al., 2005). This critical value of Si was determined for maize grown on alkaline soils in Pakistan as it can contribute upto 0.1 to 10% of the dry matter of plants (Jarvis, 1987; Epstein, 1994; Liang et al., 2005). Si-deficient plants are much more different from Si-enriched plants in structure, chemical composition, mechanical strength, yield and yield contributing factors, enzymatic activities, disease and pest resistance, metal toxicity, salt and drought tolerance etc. (Epstein, 1994).

The data regarding extractable Si concentration in surface soil samples reflected a normal depiction of Si availability in the surveyed soils and exhibited that all samples had adequate amount of Si in soils of District Okara and Tehsil Deepalpur (Table 3.4). A calibrated soil test for a particular nutrient needed for plant growth and development indicated the degree of the

deficiency of the nutrient and the amount of that nutrient to be applied as a fertilizer to correct the deficiency. Based on preliminary soil test procedures, soils containing 10 mg L⁻¹ or less of 0.5 M acetic acid extractable Si generally require Si fertilization to provide a shoot Si concentration of 30 g kg⁻¹ to produce maximum grain yield; whereas, those containing 25 mg L⁻¹ of Si or more, generally do not require Si fertilization (Elliott and Synder, 1991).

3.5 Conclusion

Farmers do not use salt affected soils to grow maize in these areas, so we cannot get any toxic effects of Na or deficiency (Si) in our samples. Soil and plant samples of district Okara presented higher concentration of Si relative to district Sargodha samples. Soil pH was higher in both maize growing areas i-e district Sargodha and Okara. District Okara samples have higher EC relative to district Sargodha which leads to the lower K Concentration in maize plant samples. District Okara plant samples have higher Na/K ratio relative to district Sargodha.

Chapter 4

Screening and characterizing maize genotypes for salinity stress tolerance at germination

Abstract

Genotypic variation exists among crop species and cultivars against salt stress. The present study was conducted to categorize the latest maize cultivars according to their tolerance against salinity stress. Initially 15 maize cultivars (Supraseed-4444, Monsento-919 and 6789, Syngenta-8441, Pioneer-30R50, Neelum, ICI hybrid, Dekalb, Golden cross, 32B33, EV-77, 1089 and 6089, Agaiti-2000 and 85) were collected and seeds were germinated in petri plates under control and 90 mM NaCl salinity. Cultivars were categorized as sensitive, medium and tolerant to salinity on the basis of performance at saline treatment relative to control. Four cultivars (Monsento-919, Golden cross, 32B33 and EV-1089) were categorized as salt sensitive, while four cultivars (Syngenta-8441, Pioneer-30R50, ICI hybrid and Dekalb) were categorized as salt tolerant.

4.1 Introduction

Excess of soluble salts in soil solution significantly decreases plant growth and yield. Poor germination of plant is the main reason of salinity stress; as the first phase of salinity stress on plant growth is physiological drought (Munns et al., 2006). Salinity delays the onset, reduces the rate and increases the dispersion of germination events (Mohammed et al., 2002), resulting in a reduced plant growth and final crop yield (Ashraf and Foolad, 2005). This low germination is also related to salinity induced disturbance of metabolic processes in crop roots leading to increase in phenolic compounds (Ayaz et al., 2000). The crop germination decreases from 30-53% with application of water EC of 9.26-16.28 dS m⁻¹ among various barley cultivars (Hussain et al., 1997). An obvious reduction was reported in radicle, plumule and seedling length in different maize varieties when they were subjected to salt stress (Farsiani and Ghobadi, 2009; Gholamin and Khayatnezhad, 2010; Khayatnezhad et al., 2010; Khodarahmpour, 2012). Poor germination leads to reduced growth and ultimately a significant yield reduction is observed (Kulkarni and Deshpande, 2007).

Crop species and cultivars within species significantly differ in their response to soil salinity (Nasim et al., 2008; Tahir et al., 2011). Growth and yield of glycophytes such as maize is severely reduced when grown in salt affected soils (Khan et al., 2006); which is a characteristic of arid to semi-arid climates. These variations can be exploited to increase crop growth and yield and to improve salinity tolerance. The primary objective of the present study was to compare latest fifteen maize cultivars towards salinity stress and to select the most salt tolerant and most sensitive cultivars of maize

4.2 Materials and methods

The study was conducted under controlled conditions in Petri plates to screen out the maize cultivars on the basis of salinity stress and later on to characterize them as most tolerant and sensitive. Seeds of fifteen maize cultivars (Table 4.1) were thoroughly washed with distilled water and rinsed into sodium hypochlorite solution for 5 minutes. Petri plates were also washed by distilled water and then autoclaved. Temperature of the growth room was maintained at 25 ± 2 . Filter paper was placed in each petri plate and 10 seeds per petri plate were placed and then covered by filter paper. Sodium chloride solution 90 mM was applied as a salinity stress to the recommended petri plates and distilled water was given to control. All treatments were fully crossed among themselves; then, petri plates were randomized and each treatment at petri plate level was replicated three times. Daily germination count was recorded. Seeds were considered germinated when the emergent radicle reached 2 mm length. After 7 days, germination percentage was measured by ISTA (International Seed Testing Association) standard method. At end of the 7th day, the length of radicle and plumule of seeds, seedling length, the germination percentage, germination index, and seed vigour (Ellis and Robert, 1981) were also measured.

$$\text{Germination percentage (GP)} = \text{SNG/SN0} \times 100$$

Where SNG is the number of germinated seeds, and SN0 is the total number of experimental seeds with viability (Scott et al., 1984).

Germination index was calculated using the following formula:

$$\text{Germination index (GI)} = \sum (Gt/Tt),$$

where Gt is the number of seeds germinated on day t and Tt is the total number of days.

$$\text{Vitality index (VI)} = S \times \text{GI}$$

Where S is the length of seedlings and GI is the germination index.

Mean Seed vigor index was calculated by the given formula given below:

$$\text{Seed vigor} = \text{Germination percentage} \times \text{Seedling length.}$$

Statistical analysis

The data obtained from this experiment was statistically analyzed by Microsoft Excel 2007® (Microsoft Cooperation, USA) and characterization made on the basis of (mean-standard deviation) and (mean + standard deviation).

4.3 Results

There was significant genotypic variation among maize cultivars in response to salinity stress (Table 1). Salinity stress significantly reduced all germination parameters irrespective of the cultivars. Categorization of maize cultivars was done on the basis of their index scores of various parameters into low, medium and high scoring cultivars. Classification is based on the relative values of each cultivar with the population mean (μ) and standard deviation (SD) for each parameter as in Aziz et al. (2011). The cultivars are assigned as low if their mean is $< \mu - \text{SD}$, medium if their mean is between $\mu - \text{SD}$ to $\mu + \text{SD}$ and high if cultivar mean is $> \mu + \text{SD}$.

Cultivars ‘EV1089’, ‘Golden Cross’ and ‘Monsento 919’ produced radical length having relative value $< 0.40 \text{ cm plant}^{-1}$ and gained lowest index score (1) when grown with applied salinity (**Error! Reference source not found.**). Cultivars ‘Dekalb’, ‘Syngenta 8441’, and ‘ICI hybrid’ gained aximum index score (3) as they produced radical length having relative value $> 1.54 \text{ cm plant}^{-1}$. Other ten cultivars were categorized as medium for radical length. Their radical length ranged from $0.43 \text{ cm plant}^{-1}$ to $1.44 \text{ cm plant}^{-1}$.

Plumule length was significantly decreased by salt stress. Five cultivars ‘EV1089’, ‘Golden Cross’, ‘32B33’, ‘Monsento 6789’, and ‘Monsento 919’ gained minimum index score (1) and relative value of plumule length was $< 0.18 \text{ cm plant}^{-1}$. Only 2 cultivars “Agaiti 85” and

“Syngenta 8441” produced plumule length >0.91 cm plant⁻¹. Remaining eight cultivars were ranged from 0.57-0.79 cm plant⁻¹.

Cultivars, ‘EV1089’, ‘32B33’, ‘Golden Cross’ and ‘Monsento 919’ were categorized as salt sensitive as they gained lowest index score (1) and produced seedling length having relative values <0.35 cm plant⁻¹, vitality index <0.24 plant⁻¹ and seed vigor index <0.26 plant⁻¹, respectively. Cultivars, ‘Dekalb’, ‘Syngenta 8441’, ‘ICI hybrid’ and Pioneer 30R50 were categorized as salt tolerant as they gained maximum index score (3) and germination % age having relative value >1.10 . Cultivars, ‘Dekalb’, ‘Syngenta 8441’ and ‘ICI hybrid’ also shown maximum vitality index having relative values >1.12 , seed vigor >1.23 and mean germination time >1.07 , respectively.

On the basis of above mentioned results, eight maize cultivars (four salt sensitive and four salt tolerant) were selected on the basis of their index scores of various parameters into low and high scoring cultivars and response of added silicon on germination of salt stressed cultivars were interpreted.

Table 4.1 Categorization of maizecultivars on the basis of their index scores of various parameters into low, medium and high scoring cultivars. Seeds were grown with sodium chloride solution in Petri plates for 8 days. Classification of each cultivar is based on performance of saline treatment relative to its control with the population mean (μ) and standard deviation (SD) for each parameter. The cultivars are assigned as low if their mean is $<\mu-SD$, medium if their mean is between $\mu-SD$ to $\mu+SD$ and high if cultivar mean $>\mu+SD$.

| Parameter | Low (Score 1) | Medium (Score 2) | High(Score 3) |
|-------------------------------|--|--|--|
| Radicle length (cm) | <0.40 EV1089<Golden Cross <Monsento919 | 0.40-1.54 Pioneer30R50<monsento6789<Supraseed4444 <Neelum<Agaiti2000<EV6089<Agaiti85<EV77<32B33 | >1.54 Dekalb< Syngenta8441< ICI hybrid |
| Plumule length (cm) | <0.18 GoldenCross<Monsento919 <32B33<EV1089<Monsento6789 | 0.18-0.91 Dekalb<ICI hybrid<EV77<Agaiti2000<EV6089 <Pioneer30R50<Neelum<Supraseed4444 | >0.91 Agaiti85< Syngenta 8441 |
| Seedling length (cm) | <0.35 EV1089<Monsento919 <Golden Cross<32B33 | 0.35-1.20 Syngenta8441<Supraseed4444<Pioneer30R50 <Monsento6789<Agaiti2000<Neelum <EV6089<Agaiti85 | > 1.20 Dekalb<ICI hybrid<EV77 |
| Germination percentage | <0.71 32B33<Monsento919 <Golden Cross | 0.71-1.10 Pioneer30R50< Supraseed 4444< EV6089=Agaiti85 <Agaiti 2000 <EV1089 <Monsento6789 < EV77< Neelum | > 1.10 Syngenta8441<ICI hybrid<Dekalb |
| Vitality index | <0.24 Monsento919<Golden Cross <EV1089< 32B33 | 0.24-1.12 Supraseed 4444< Pioneer30R50< EV6089<Agaiti85 < Agaiti 2000< Monsento6789<Neelum | >1.12 Syngenta8441<ICI hybrid=Dekalb <EV77 |
| Seed vigour index | <0.26 Monsento919<Golden Cross <32B33<EV1089 | 0.26-1.23 EV77< Supraseed 4444< Pioneer30R50 <Monsento6789<Agaiti 2000<EV6089 <Agaiti85<Neelum | >1.23 Dekalb< Syngenta8441< ICI hybrid |
| Mean germination Time | <0.97 Dekalb< Syngenta8441< ICI hybrid | 0.97-1.07 Monsento6789=Monsento919< Pioneer30R50=Golden Cross<Neelum<Agaiti2000 <Agaiti85=Supraseed4444<EV77 | >1.07 32B33<EV1089=EV6089 |

4.4 Discussion

Soil salinity significantly affects seed germination and early growth (Misra and Dwivedi, 2004) by decreasing germination rate and initiation of seedling growth (Almansouri et al., 2001). Significant reduction in seedling length under salinity has been reported in maize and it has been categorized as salt sensitive crop particularly at germination stage (Farsiani and Ghobadi, 2009; Gholamin and Khayatnezhad, 2010; Khayatnezhad et al., 2010). In present experiment, germination of all cultivars reduced significantly because of salinity stress (Table 1), however the effect was different among cultivars.

The absolute values of seedling length, vitality index, seed vigor of salt sensitive cultivars such as EV1089 and 32B33, were more or less similar or even higher than salt tolerant cultivars, but their performance at saline treatments was very poor (Table 1). Hence cultivars were categorized for their performance at stress conditions relative to their potential yield/growth at normal conditions. So the categorization of different maize cultivars was based on the performance of saline treatment relative to its control with the population mean (μ) and standard deviation (SD) for each parameter following Aziz et al. (2011) who categorized the brassica cultivars against phosphorus deficiency stress. Cultivars 'Syngenta 8441', 'Dekalb', and 'ICI hybrid' were efficient against salt stress (Table 1) in all parameters studied, while cultivars '32B33', 'EV 1089', and 'Golden Cross' were sensitive to NaCl stress.

Salinity stress generally delays seed germination (Mohammed et al., 2002), which was observed by increased mean germination time of all cultivars under salinity stress, however cultivars varied for mean germination time (Table 1). Decreased osmotic potential in saline treatment (Munns et al., 2006) is one of the reasons for increased mean germination time in corn (Alebrahim et al., 2008). Salinity stress caused a decrease in seed vigor with a significant inter-genotype variation (Table 1).

4.5 Conclusion

There was significant genotypic variation found among different maize cultivars with the application of NaCl. Mean germination time increased in all cultivars with NaCl stress. Seedling length was also significantly reduced in salt sensitive cultivars (Monsento-919, Golden cross,

32B33 and EV-1089) as compared to salt tolerant (Syngenta-8441, Pioneer-30R50, ICI hybrid and Dekalb).

Response of selected maize genotypes to silicon application under salinity stress at germination

Abstract

Poor seed germination is the major concern in soils having salinity problem. Better germination and seedling establishment may result in better economic yields. Silicon uptake in salt stressed plant increases root activity for nutrient uptake and reduces osmotic stress. The main objective of the present study was to screen out the selected salt tolerant and sensitive maize cultivars at different growth stages on the basis of their Si uptake ability. In previous experiment, Four cultivars (Monsento-919, Golden cross, 32B33 and EV-1089) were categorized as salt sensitive, while four cultivars (Syngenta-8441, Pioneer-30R50, ICI hybrid and Dekalb) were categorized as salt tolerant. These eight cultivars were selected and seeds were germinated in petri plates with 90 mM NaCl and 2 mM K₂SiO₃. Salinity stress significantly decreased all of germination parameters while Si application improved those parameters however effect was variable among tolerant and sensitive cultivars. Silicon application significantly increased seedling length of two salt sensitive cultivars, 'EV1089' and '32B33' under salinity stress; while salt tolerant cultivar 'ICI hybrid' produced radical length of 2 and 3 folds when Si was applied under salinity stress relative to saline treatment. Availability of Si in plant tissues reduced Na⁺ uptake by improving all germination parameters and reducing mean germination time. This study implies that Si application can enhance the seedling growth and seed germination of maize under salinity stress.

5.1 Introduction

Salinity has more than an instantaneous effect on the plant physiology, influencing the differentiation of the xylem vessels, and confirming the importance of long-term studies on the response of glycophytes to salinity (Flowers and Colmer, 2015). Salinity delays the onset, reduces the rate and increases the dispersion of germination events (Mohammed et al., 2002), resulting in a reduced plant growth and final crop yield (Ashraf and Foolad, 2005). This low

germination is also related to salinity induced disturbance of metabolic processes in crop roots leading to increase in phenolic compounds (Ayaz et al., 2000).

Silicon is known to improve plant growth particularly under abiotic stresses. It improves plant water status in context of relative water content and transpiration rate (Romero-Aranda et al., 2006; Tahir et al., 2011), ameliorates the harmful effects of salinity on chlorophyll content and plant biomass (Tuna et al., 2008), it lowers significantly the Na^+ concentrations in both leaves and roots (Liang et al., 2003; Kafi and Rahimi, 2011; Tahir et al., 2012), improves leaf erectness, decreases susceptibility to lodging, prevents manganese, cadmium or iron toxicity, decreases the influence of leaf pathogens (Marchner, 1995).

Most of these studies reported the beneficial effects of Si on growth of crop plants at vegetative growth and at maturity. As germination and early vegetative growth is very important for good crop stand and growth, hence it is very important to study the beneficial effects of Si at germination stage and to screen maize germplasm for salinity tolerance at germination. We hypothesized that Si application can improve maize germination and early vegetative growth under salinity stress. The primary objective of the present study was to comparing selected eight cultivars against salinity and silicon treatments to study the role of Si in improving maize germination parameters.

5.2 Materials and methods

Four salt sensitive maize cultivars (Monsento-919, Golden cross, 32B33 and EV-1089) and four salt tolerant (Syngenta-8441, Pioneer-30R50, ICI hybrid and Dekalb) were selected on the basis of their index scores of various parameters into low, medium and high scoring cultivars from experiment 1 to study the response of added silicon on germination. Seeds were thoroughly washed by distilled water and rinsed into sodium hypochlorite solution for 5 minutes. Petri plates were also washed by tap water and then autoclaved. Temperature of the growth room was maintained at 25 ± 2 . After wards, filter paper was placed in each petri plate (10 cm diameter) and 10 seeds per petri plate were sown and then covered by filter paper. There were two salinity levels viz 0 and 90 mM NaCl and two Si levels viz 0 and 2 mM added through spray. Silicon was added as potassium silicate (K_2SiO_3). Treatments were fully crossed among themselves; then, petri plates were randomized and each treatment at petri plate level was replicated three

times. All treatments were arranged in triplicate according to CRD (Completely Randomized Design) with three way ANOVA experiment.

Daily germination count was recorded. After 8 days, following parameters were recorded.

- a) Radicle length (cm)
- b) Plumule length (cm)
- c) Vitality index
- d) Seedling length (cm)
- e) Seed vigor index

Vitality index can be calculated as

$$\text{Vitality index (VI)} = S \times \text{GI}$$

Where S is the length of seedlings and GI is the germination index.

Mean Seed vigor index was calculated by the given formula given below:

$$\text{Seed vigor} = \text{Germination percentage} \times \text{Seedling length.}$$

5.3 Results

Radical length in various maize cultivars ranged from 2.4 to 21.2 cm at various rates of NaCl and Si. All the main effects, cultivar \times NaCl application interaction and NaCl \times Si application interaction significantly ($P \leq 0.01$) affected radical length (Figure 5.1). Salinity significantly reduced radical length of salt sensitive cultivar 32B33, while 8 fold increases was observed with Si application under salinity stress. However, genotypes also differed significantly ($P \leq 0.01$) with NaCl application not only on average bases but also in their response to Si application. Pioneer 30R50 and ICI hybrid produced radical length of 2 and 3 folds when Si was applied under salinity stress relative to saline treatment.

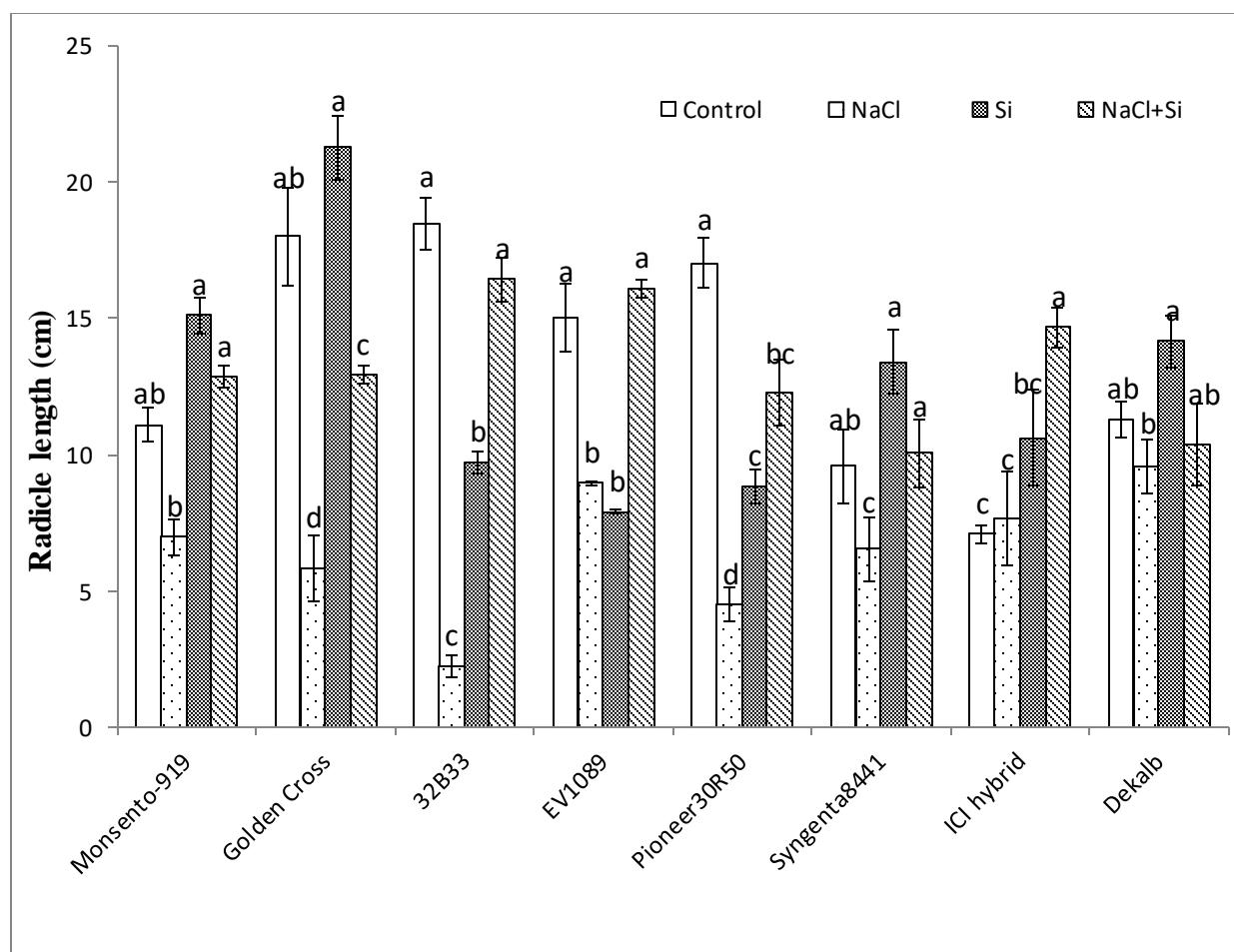
The plumule length of maize cultivars was significantly ($P \leq 0.01$) influenced by main and interactive effects of NaCl and Si applications (Figure 5.2). On average, salinity stress decreased the plumule length while Si application under salinity stress significantly

increased plumule length. Cultivar, 'EV1089' showed no plumule growth when salt stress was applied. Maximum plumule length was observed in Monsento 919 (9.6 cm) while minimum plumule length in EV-1089 (0 cm). Silicon application under salinity stress increased 55 and 43% in plumule length relative to saline treatment.

There were significant ($P \leq 0.01$) main and interactive effects of salinity and Si applications on seedling growth (Table 5.1). Salinity stress significantly decreased the seedling length in all cultivars. However, cultivars differed significantly ($P \leq 0.01$) in their response to the salinity and Si application. There were 53 and 86 % increase in seedling length observed of two salt sensitive cultivars, 'EV1089' and '32B33' with Si application under salinity stress compared to saline treatment.

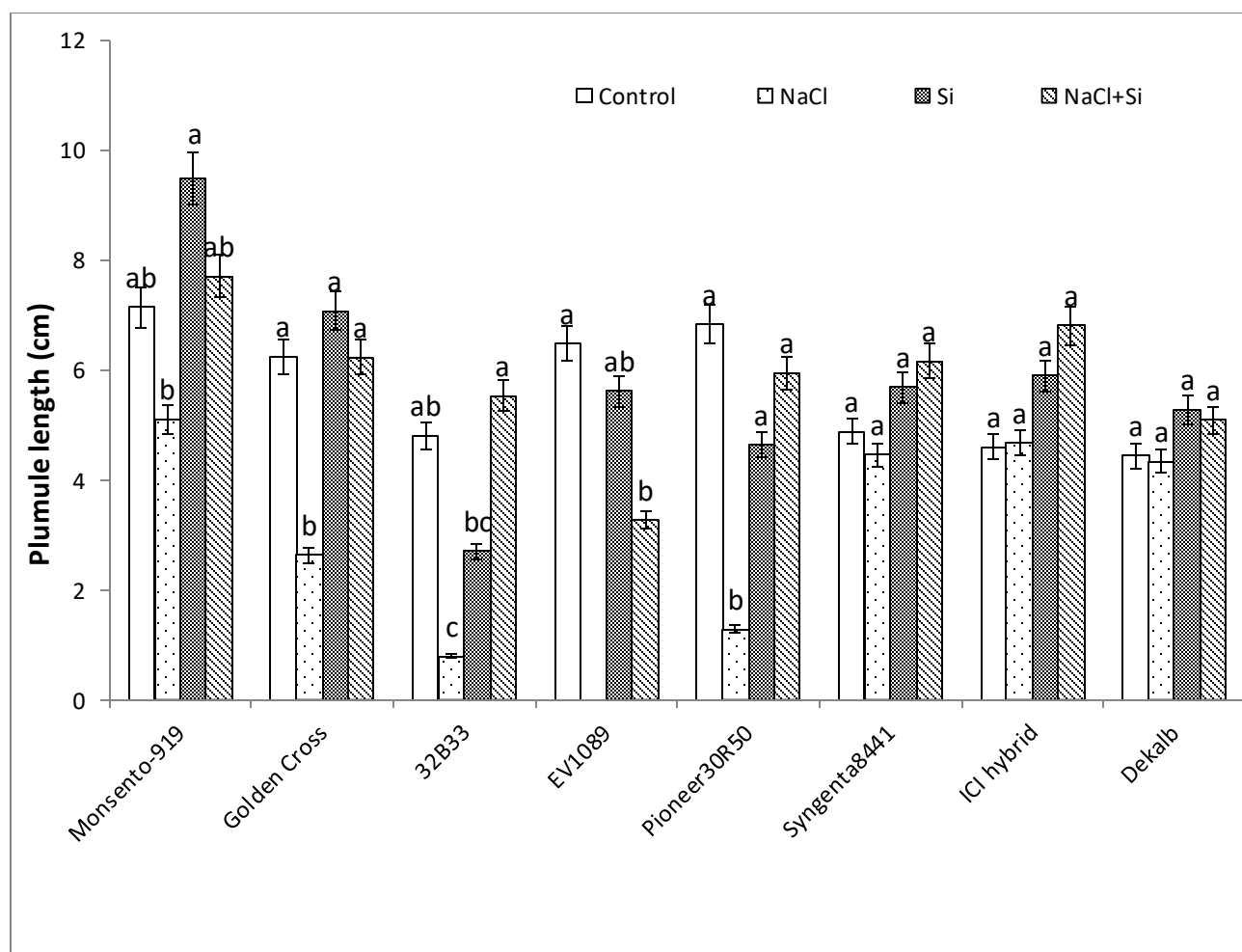
Seed vigor index of maize cultivars was significantly ($P \leq 0.01$) influenced by main and interactive effects of cultivars, salinity and Si application (Table 5.2). In general, application of Si increased while salinity stress decreased the seed vigor index in salt sensitive cultivars. The application of Si did not affect the seed vigor index in two cultivars, 'Syngenta 8441' and 'Dekalb' under salinity, while it improved seed vigor in rest of 6 cultivars. .

Vitality index of maize cultivars was significantly ($P \leq 0.01$) influenced by interactive effects of cultivar, salinity and Si application (Figure 5.3). On average, vitality index was decreased about 6 folds because of salinity. Silicon application significantly increased vitality index in all maize cultivars; while '32B33' and 'ICI hybrid' showed maximum vitality index of 211 and 179, respectively.



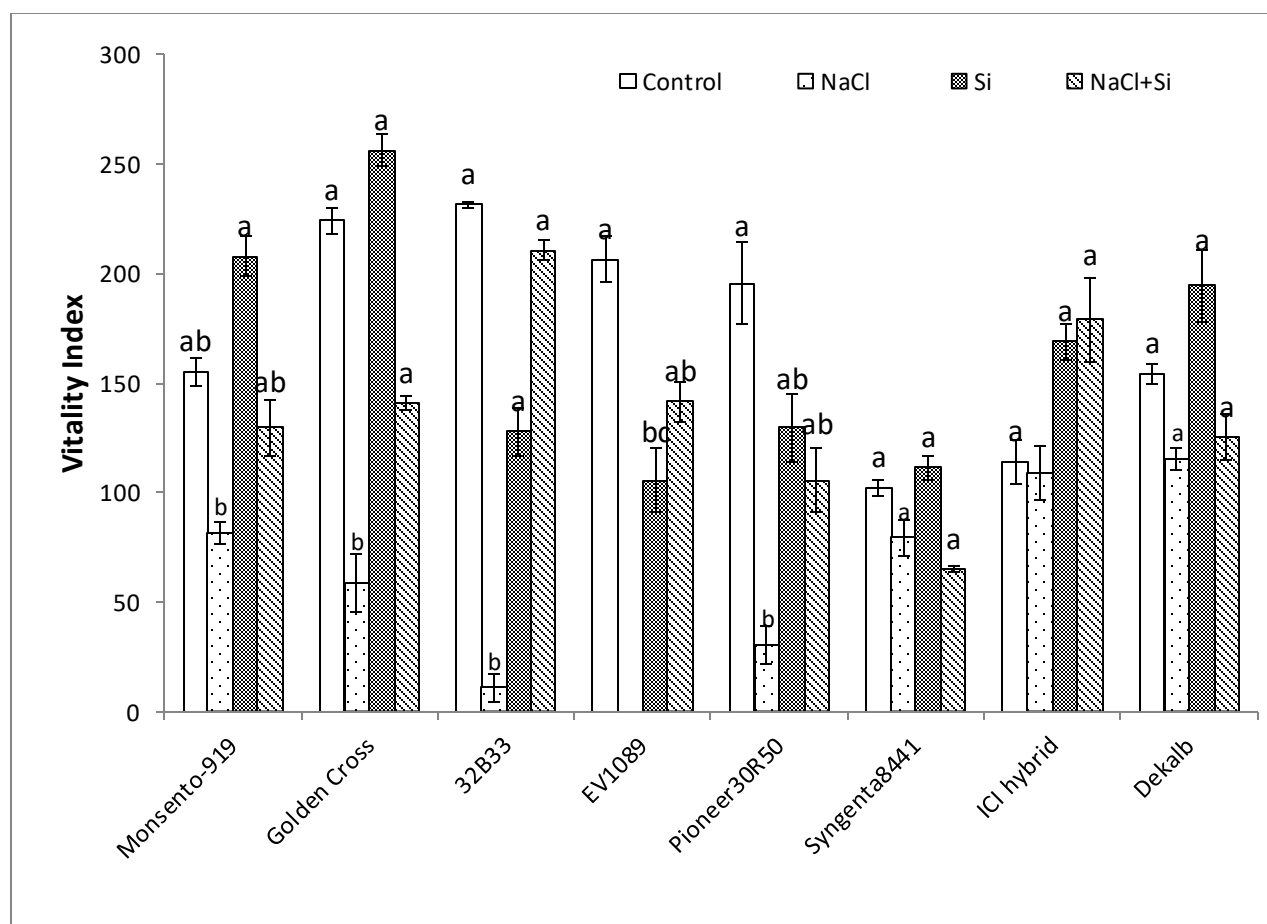
(LSD0.01 for three way interaction = 3.03)

Figure 5.1 Radical length (cm) of eight maize cultivars supplied with different rates of salinity (0 and 90 mM NaCl) and Si (0 and 2 mM K_2SiO_3). The seeds were germinated in petri-plates. Letters belong to each cultivar separately and values are mean \pm S.E.three replicates. Cultivar '32B33' produced minimum radical length under salt stress and regarded as salt sensitive while 'Dekalb' produced maximum radicle length and regarded as salt tolerant cultivar.



(LSD0.01 for three way interaction = 2.03)

Figure 5.2 Plumule length of eight maize cultivars supplied with different rates of of salinity (0 and 90 mM NaCl) and Si (0 and 2 mM K_2SiO_3). The seeds were germinated in petri-plates. Letters belong to each cultivar separately and values are mean \pm S.E.of three replicates. Cultivar ‘EV1089’ produced minimum no plumule under salt stress and regarded as salt sensitive while ‘Syngenta 8441 and Monsento 919’ produced maximum radicle length and regarded as salt tolerant cultivars.



(LSD0.01 for three way interaction = 48.4)

Figure 5.3 Vitality index of eight maize cultivars supplied with different rates of salinity (0 and 90 mM NaCl) and Si (0 and 2 mM K_2SiO_3). The seeds were germinated in petri-plates. Letters belong to each cultivar separately and values are mean \pm S.E. of three replicates. Cultivar 'EV1089' showed no vitality index under salt stress and regarded as salt sensitive while 'ICI hybrid and Dekalb' showed maximum vitality index and regarded as salt tolerant cultivars.

Table 5.1 Seed vigor index of eight maize cultivars supplied with different rates of salinity (0 and 90 mM NaCl) and Si (0 and 2 mM K₂SiO₃). There were six seeds per petri plate and values are mean± S.E.of three replicates.

| Cultivars | Control | NaCl | Si | NaCl+Si |
|---------------------|----------------|-------------|-----------|----------------|
| Monsento-919 | 16.4±0.45 | 8.61±1.14 | 23.4±1.41 | 14.5±1.33 |
| Golden Cross | 22.8±1.67 | 5.12±0.61 | 25.6±1.08 | 17.1±1.0 |
| 32B33 | 23.2±1.37 | 1.48±0.50 | 12.4±1.99 | 21.9±0.38 |
| EV1089 | 21.4±1.61 | 0 | 11.2±1.48 | 16.4±1.08 |
| Pioneer30R50 | 23.4±0.99 | 4.03±0.17 | 13.5±2.32 | 15.5±0.98 |
| Syngenta8441 | 11.9±0.91 | 8.82±1.01 | 14.8±1.90 | 10.6±0.41 |
| ICI hybrid | 11.2±1.56 | 11.1±2.04 | 15.6±1.29 | 18.4±2.10 |
| Dekalb | 15.7±1.47 | 12.6±1.43 | 19.4±1.51 | 14.7±1.55 |

Table 5.2 Seedling length (cm) of eight maize cultivars supplied with different rates of salinity (0 and 90 mM NaCl) and Si (0 and 2 mM K₂SiO₃). There were six seeds per petri plate and values are mean± S.E.three replications.

| Cultivars | Control | NaCl | Si | NaCl+Si |
|---------------------|----------------|-------------|------------|----------------|
| Monsento-919 | 18.2±1.11 | 12.06±1.03 | 24.56±0.42 | 20.5±0.42 |
| Golden Cross | 24.2±2.57 | 8.46±2.61 | 28.3±0.69 | 19.14±0.69 |
| 32B33 | 23.2±1.37 | 3.04±0.80 | 12.4±1.99 | 21.9±1.99 |
| EV1089 | 21.4±1.61 | 8.95±0.05 | 13.5±1.65 | 19.35±1.65 |
| Pioneer30R50 | 23.8±2.22 | 5.79±0.83 | 13.46±2.32 | 18.24±2.32 |
| Syngenta8441 | 14.4±2.48 | 10.9±1.39 | 19.08±1.62 | 16.21±1.62 |
| ICI hybrid | 11.6±1.15 | 12.3±2.24 | 16.49±1.79 | 21.46±1.79 |
| Dekalb | 15.7±1.47 | 13.8±0.89 | 19.45±1.51 | 15.44±1.51 |

5.4 Discussion

Soil salinity is detrimental in plant life as negatively affecting metabolic and physiological processes eventually reducing yield, growth and production of agronomic crops (Ashraf and Harris, 2004). Radical and plumule length are important traits regarding salinity stress. An obvious reduction was reported in radicle, plumule and seedling length different maize varieties (Figure 5.1, 5.2; Table 5.1) when they were subjected to salt stress (Farsiani and Ghobadi, 2009; Khayatnezhad et al., 2010). This low germination is also related to salinity induced disturbance of metabolic processes leading to increase in phenolic compounds (Ayaz et al., 2000). The crop germination decreased between 24-35% with irrigation water having EC of 9.26 dS m⁻¹, 28-47% with water EC of 13.4 dS m⁻¹ and 30-53% with water EC of 16.28 dS m⁻¹ among various barley cultivars (Hussain et al., 1997).

Mineral nutrition is an effective strategy to increase salt resistance in plants and to sustain crop productivity in low input and environmental friendly agriculture systems (Tuna et al., 2008). Chemical treatments can stimulate seeds germination in many plant species (Meot-Duros and Magne, 2008). As reactive oxygen species production and lipid peroxidation are the major consequences of salt stress which further deteriorates the seed (Lehner et al., 2008), so exogenous application of ethanol and ascorbic acid can protect the seed against reactive oxygen species and lipid peroxidation, thus enhancing seed germination. In our experiment, the exogenous Si application in growth medium increased the seed vigor index, vitality index, the length of radical and plumule in maize under salt stress (Table 5.1; Figure 5.1, 5.2, 5.3). These findings suggested that Si may be involved directly or indirectly in both morphological changes and physiological processes in plants (Moussa, 2006). Silicon is involved in strengthening the plants against oxidation of cell membranes, leading to the protection of various plant organs subjected to salt stress conditions (Epstein, 1999). Silicon also appears to be part of the regulation of osmolytes within cells subjected to salt stress (Epstein, 1999). In most cases, Si does not appear to be beneficial to plants until some stress is imposed (Ma and Yamaji, 2008).

Since salinity in the rhizosphere is often associated with water deficit (Taiz and Zeiger, 2008), there are some reports about a protective role of Si in seed germination of plant species to prevent them from being severely affected by salt stress (Sedghi et al., 2010). Under saline condition, the excellent performance of Si-treated EV-1089 than without addition of Si can be

associated with regulation on other mineral nutrition (N, P, Ca and K) uptake, which required to be further investigated.

5.5 Conclusion

There was significant genotypic variation found among different maize cultivars undersalt stress. Seed vigor index decreased among all cultivars under salt stress. Silicon application enhanced all the germination parameters when applied in combination with NaCl. Seed vigor index and vitality index were significantly increased in two salt sensitive cultivars, 'EV1089' and '32B33' with Si application under salinity stress; while salt tolerant cultivar 'ICI hybrid' produced radical length of 2 and 3 folds when Si was applied under salinity stress relative to saline treatment. On the basis of alone NaCl, interactive effects of Si and NaCl, seedling growth and germination parameters; Syngenta-8441 was categorized as salt tolerant and EV-1089 as salt sensitive cultivars.

Chapter 6

Response of maize genotypes to silicon application under salinity stress at early vegetative growth stage

Abstract

The cultivars used in previous experiment were screened against salinity stress at early vegetative growth. The objective of this study was to test different cultivars in soil conditions to evaluate different ionic parameters. Plants were grown with two Si levels (0 and 2 mM) and two levels of salinity (0 and 60 mM NaCl). Each treatment was replicated three times. Salt stress significantly reduced shoot K and Si concentration; but the difference remained variable among salt tolerant and salt sensitive cultivars. Shoot K and Si concentration was significantly reduced in salt sensitive cultivar (EV 1089) as compared to salt tolerant (Syngenta 8441) under salt stress. Silicon treated maize plants perform far better than non-treated plants in saline conditions. Silicon application increased the K concentration in maize shoot. Significant genetic variation existed among genotypes in present study. Genotype Syngenta 8441 performed better than the other cultivars in all parameters under salinity stress.

6.1 Introduction

Saline soils impose a physiological challenge to the plants; as soil pore water has highly negative water potential leading to reduced water availability than non-saline habitats (Reef and Lovelock, 2015). The ability of plants to maintain water uptake in saline conditions is key to salt tolerance. Salt stress has a number of deleterious effects on seed germination, seedling growth and vigor, vegetative growth, flowering, fruit set, activities of enzymes, integrity of cellular membrane, and functioning of the plant photosynthetic apparatus (Cheeseman, 1988; Shannon, 1997; Flowers, 2004; Sairam and Tyagi, 2004). The major reason of reduced growth of cereal crops in salt stressed condition is specific ion toxicity (certain ions like Na and Cl uptake at elevated level) (Chinnusamy et al., 2005; Tahir et al., 2012). At early growth stage, different salt treatments affect plants in a different way, at varying levels of salt, if germination starts and seeds are emerged, still their development could not be continued. Salinity stress poses a

significant decrease in shoot fresh and dry weights, its length, and leaf area with the incremental rates of salinity (Nuran and Cakirlar, 2002).

Judicious use of mineral nutrition is also a healthy strategy as it strengthens the plants to cope with salt stress. In plants; Si is classified as a quasi-essential element (Epstein, 1999). Nevertheless, plants accumulate it in higher amounts and it can contribute upto 0.1 to 10% of the dry matter of plants. This wide variation in Si concentration in plant tissues is attributed mainly to differences in the characteristics of Si uptake and transport (Epstein, 1994; Liang et al., 2005). Silicon plays a significant role in minimizing the harmful effects of salinity stress. Enhanced H_2O_2 , free proline level and malondialdehyde concentration in plants is an indicative of salinity stress and application of Si has been reported to reduce all these parameters (Moussa, 2006). Similarly, Kafi and Rahimi (2011) reported that application of Si improved root dry weight, root area, and leaf and root K content in the presence of salinity, while leaf and root sodium (Na) content and leaf proline content were decreased. Silicon application also improves water storage within plant tissues thereby diluting the salt concentration within tissues, which allows a higher growth rate that, in turn, mitigating salt toxicity effects (Romero-Aranda et al., 2006). The other mechanisms for salinity tolerance induced by Si application are ;enhanced bioactive gibberellins (GA_1 and GA_4) contents and reduced Jasmonic acid (JA) contents of soybean leaves under salinity stress (Hamayun et al., 2010).

Maize is an important grain food crop and being glycophyte is also severely affected by the soil salinity (Khan et al., 2006). As crop yield and its sustainability is a pre-requisite to flourish the country's economy, it is highly recommended to adopt strategies aiming at increased crop production on salt affected lands. As food security is becoming a serious issue in developing countries due to salt affected lands. This problem can be solved by increasing yield per acre by the addition of mineral nutrition like Si.

To the best of our knowledge, very few studies yet reported on the interactive effect of Si and NaCl at early vegetative growth stage of maize. We hypothesized that Si application can increase maize crop growth under salt stress conditions at early growth stages which is critical under salinity stress. In our germination experiments, different maize cultivars were categorized as salt sensitive and tolerant on their Si uptake ability; now eight cultivars were tested in soil conditions to evaluate different ionic parameters.

6.2 Materials and methods

A pot study was carried out in wire-house, Institute of Soil and Environmental Sciences (ISES), University of Agriculture Faisalabad. The soil (0-15 cm) was collected from ISES research area. The collected soil was sieved through a 2.0 mm sieve. For various physico-chemical properties of soil, a sub sample of the prepared soil was analyzed for texture (Gee and Bauder, 1986), pH, electrical conductivity (EC), organic matter (Nelson and Sommer, 1982). There were two Si levels (0 and 2 mM) and two levels of salinity (0 and 90 mM NaCl). Each treatment was replicated three times.

Table 6.1 Physico-chemical properties of soil used for pot experiment

| | |
|--|------------------|
| Textural class | Clay loam |
| pH | 7.54 |
| ECe (dS m ⁻¹) | 1.13 |
| Organic matter (%) | 0.53 |
| Extractable Potassium (mg Kg ⁻¹) | 137 |
| Cation exchange capacity (cmol _c kg ⁻¹) | 6.46 |
| Calcium carbonate (%) | 3.38 |

Prepared soil was filled in each pot @ 8 Kg soil and 10 seeds were sown; each of eight selected cultivars i.e Four salt sensitive maize cultivars (Monsento-919, Golden cross, 32B33 and EV-1089) and four salt tolerant (Syngenta-8441, Pioneer-30R50, ICI hybrid and Dekalb). There were two Si (0 and 2 mM) and two levels of salinity (0 and 60 mM NaCl). Each treatment was replicated three times. Pots were randomized accordingly. After four days of germination, four plants per pot were maintained. Distilled water was used to maintain moisture contents of soil at field capacity in all pots during the experimental period. Plants were harvested after 25 days of growth.

Plant samples were washed with distilled water and blotted dry with tissue paper. The plant samples were divided into root and shoot, air-dried and then oven dried at 65°C in a forced air driven oven (WFO-600ND, Tokyo Rikakiai Co. Ltd.) to a constant weight. Dry matter yield was taken and plant samples were ground. Plant samples were tested for the Si, Na⁺ and K⁺

concentration and their contents. Finely ground plant samples (0.1 g) were digested in a di-acid ($\text{HNO}_3\text{:HClO}_4$) mixture (Jones and Case, 1990). The Na and K concentration in the digest was estimated by flame photometer (Jenway, PFP-7). For Si determination, the ground samples (0.2 g) were digested in 2 mL 50% hydrogen peroxide (H_2O_2) and 4.5 mL 50% NaOH in open vessels (Teflon beakers) on a hot plate at 150°C for 4 hours. Silicon concentration was measured using calorimetric amino molybdate blue color method (Elliot and Synder, 1991) on UV-visible spectrophotometer (Shimadzu UV-1201). To 1 mL of supernatant filtrate liquid, 10 mL of ammonium molybdate (54g L^{-1}) solution and 25 mL of 20% acetic acid was added in 50 mL of polypropylene volumetric flask.

After five minutes, 5 mL of 20% tartaric acid and 1 mL of reducing solution was added in flask and volume was made with 20% citric acid. The reducing solution was made by combining solution A (2 g of Na_2SO_3 in 25 mL of DM plus 0.5 g of l-amino-2-naphthol-4-sulfonic acid) and solution B (25 g of NaHSO_3 dissolved in about 200 mL of DM) and diluting to 250 mL. After 5 but not more than 30 minutes, the absorbance was measured at 650 nm wave length with a UV visible spectrophotometer (Shimadzu, Spectronic 100, Japan).

Statistical analysis

Data was statistically analyzed by *Microsoft Excel 2010*[®] (Microsoft Cooperation, USA) and *Statistix 8.1*[®] (Analytical Software, Tallahassee, USA). Significantly different treatment means were separated using least significant difference (LSD) test (Steel et al., 1997).

6.3 Results

6.3.1 Shoot dry weight

In general, silicon addition had no significant effect on the shoot dry weight in all cultivars as compared to salinity treatment (Table 6.1). Cultivars ‘EV-1089’ and ‘32B33’ showed 67 and 46% decrease in shoot dry weight when salinity was applied as compared to control; however Si application under salinity stress increased 48 and 52% shoot dry weight in cultivars ‘EV-1089’ and ‘32B33’ compared to plants grown under salinity stress.

6.3.2 Shoot Na concentration

There was a significant ($p < 0.05$) increase in shoot Na concentration among all cultivars with salt treatment relative to control (Figure 6.1). Silicon application decreased shoot Na concentration in all cultivars relative to saline treatment only. Cultivars 'Syngenta-8441' and 'Monsento 6789' showed 16 and 29% decrease in shoot Na concentration with Si application under salinity stress as compared to saline treatment. Maximum increase (46%) in shoot Na concentration was observed in Cultivar Syngenta-8441 with Si application under salinity stress.

6.3.3 Shoot K concentration

The K concentration in maize shoot ranged from 3.34 to 7.5 mg g⁻¹ (Figure 6.2). The main effects of salinity and Si applications and the interaction of soil NaCl application with Si application significantly ($p \leq 0.05$) influenced shoot K concentration. Salt stress significantly decreased shoot K concentration in all cultivars except Syngenta 8441. This decrease was 46 and 33% in cultivars EV 1089 and ICI Hybrid with saline treatment compared to control. But Si application under salinity stress increased shoot K concentration in all the cultivars compared to saline treatment. Cultivars 'EV 1089' and 'Monsento 6789' shown 2 fold increase in shoot K concentration with Si application under salinity stress relative to saline treatment.

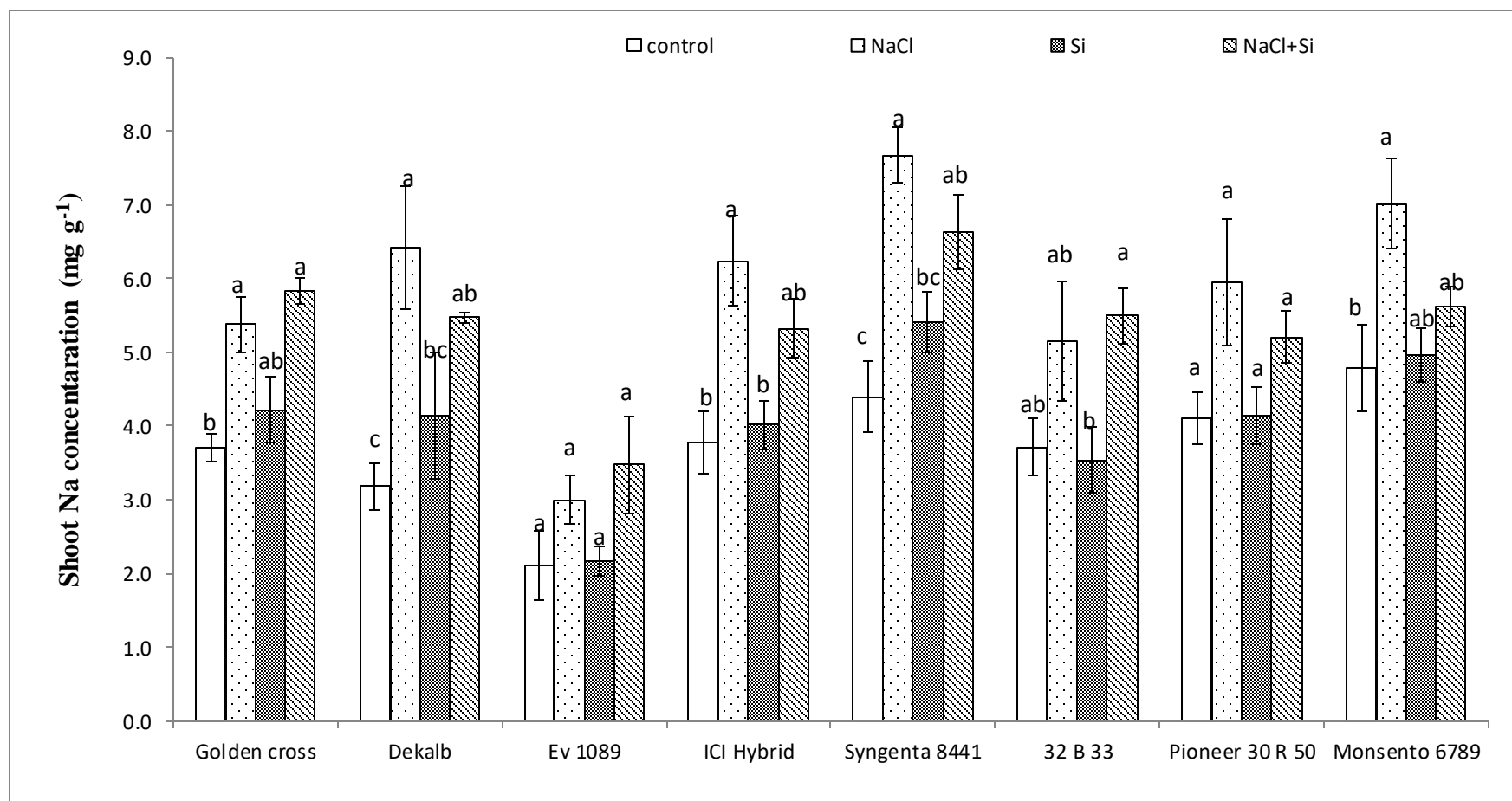
6.3.4 Shoot Si concentration

Silicon concentration in various maize cultivars ranged from 7.20 to 14.78 mg g⁻¹ (Figure 6.3). Salinity stress significantly ($p \leq 0.05$) decreased the shoot Si concentration in cultivars 'EV 1089' and 'ICI hybrid' compared to control. There was 27 and 21% decrease in shoot Si concentration observed in cultivars 'EV 1089' and 'ICI hybrid' when salinity was applied relative to control. On average, Si application under salinity stress increases the shoot Si concentration in all cultivars compared to saline treatment only. Cultivars 'Golden Cross', 'EV 1089' and 'ICI hybrid' showed 56, 33 and 24 % increase in shoot Si concentration with Si application under salinity stress relative to saline treatment.

Table 6.2 Shoot dry weight (g) of eight maize cultivars supplied with different rates of salinity (0 and 50 mM NaCl) and Si (0 and 2 mM H₂SiO₃). There were four plants per pot. Data are shown as means \pm SE of three replicates

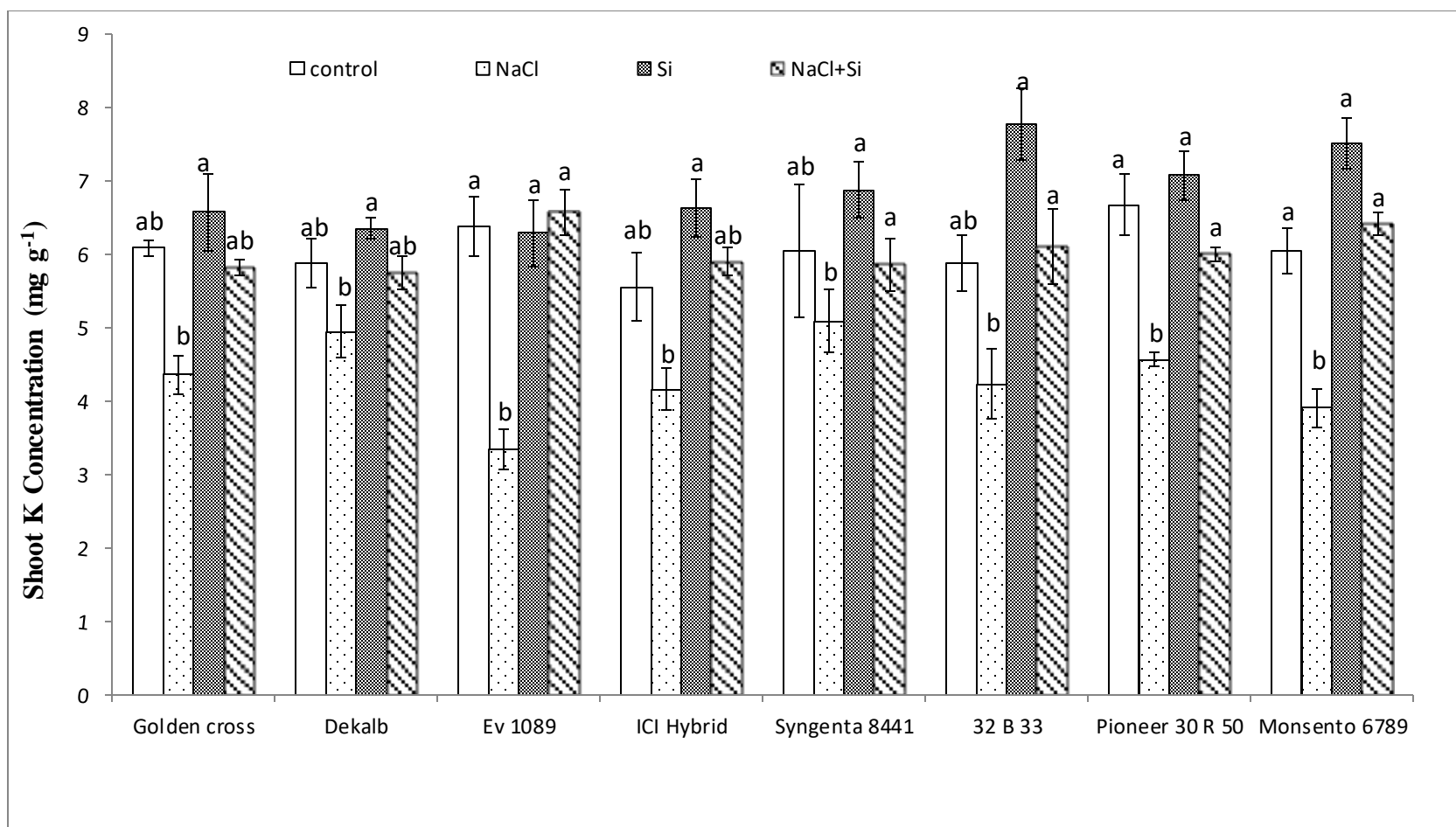
| Dry weight | | | | |
|------------------------|-----------------|-----------------|-----------------|-----------------|
| Cultivars | Control | NaCl | Si | NaCl+Si |
| Golden cross | 1.1 \pm 0.13 | 0.47 \pm 0.10 | 1.25 \pm 0.21 | 1.3 \pm 0.06 |
| Dekalb | 1.97 \pm 0.23 | 1.09 \pm 0.12 | 1.49 \pm 0.06 | 1.42 \pm 0.07 |
| EV-1089 | 1.46 \pm 0.27 | 0.47 \pm 0.12 | 1.91 \pm 0.28 | 0.92 \pm 0.1 |
| ICI Hybrid | 1.10 \pm 0.20 | 1.20 \pm 0.25 | 1.25 \pm 0.05 | 1.30 \pm 0.2 |
| Syngenta 8441 | 1.75 \pm 0.25 | 1.42 \pm 0.10 | 1.87 \pm 0.19 | 1.93 \pm 0.21 |
| 32 B 33 | 1.40 \pm 0.11 | 0.72 \pm 0.11 | 1.77 \pm 0.06 | 1.52 \pm 0.22 |
| Pioneer 30 R 50 | 1.52 \pm 0.04 | 0.69 \pm 0.18 | 1.25 \pm 0.13 | 1.08 \pm 0.27 |
| Monsento 6789 | 1.19 \pm 0.26 | 0.79 \pm 0.19 | 1.44 \pm 0.11 | 2.2 \pm 0.28 |

(LSD_{0.01} for three way interaction= **0.95**)



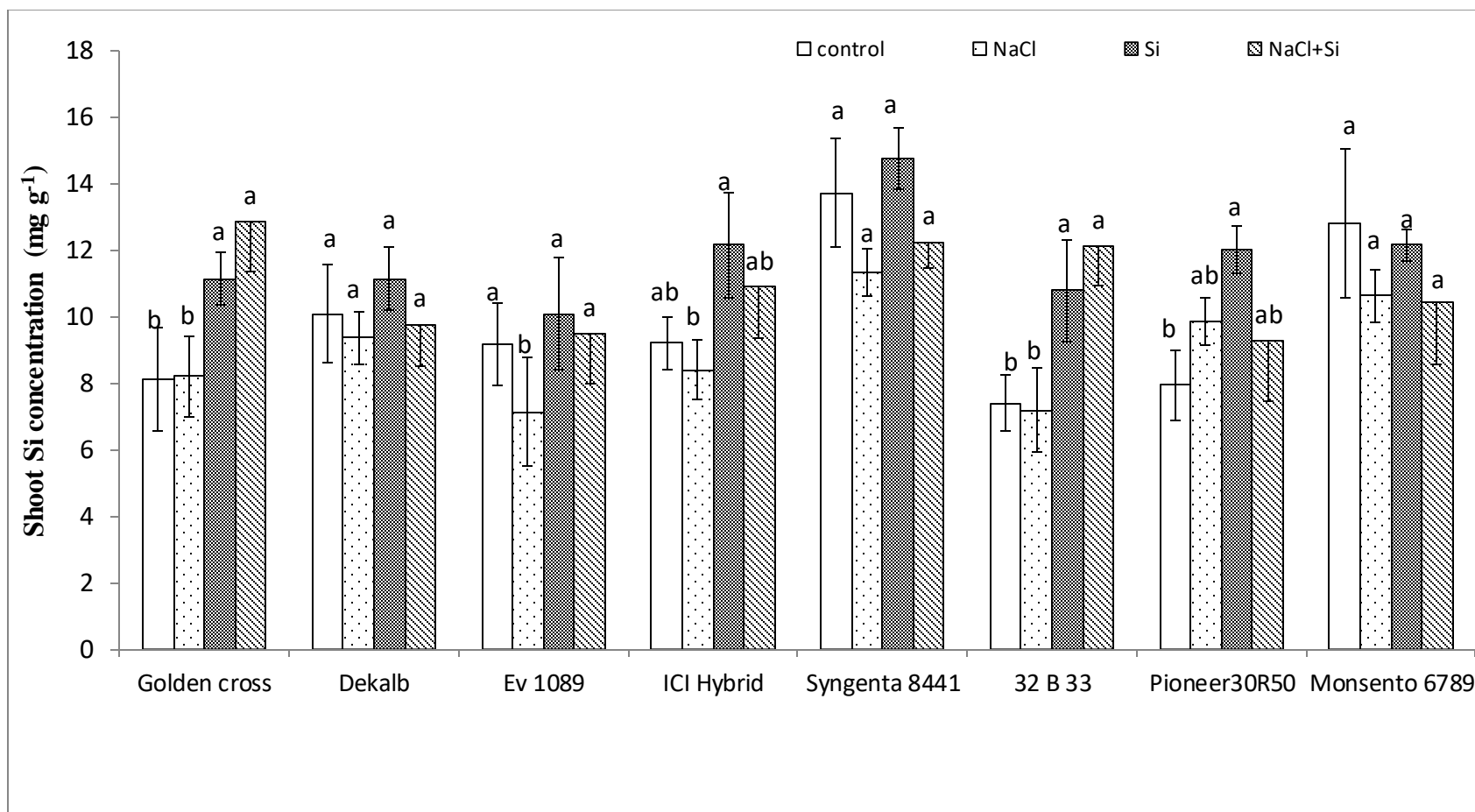
(LSD_{0.01} for three way interaction= 1.9)

Figure 6.1 Shoot Na concentration (mg g^{-1}) of eight maize cultivars supplied with different rates of salinity (0 and 50 mM NaCl) and Si (0 and 2 mM H_2SiO_3). There were four plants per pot. Letters belong to each cultivar separately and data are shown as means \pm SE of three replicates.



(LSD_{0.01} for three way interaction= 2.02)

Figure 6.2 Shoot K concentration (mg g^{-1}) of eight maize cultivars supplied with different rates of salinity (0 and 50 mM NaCl) and Si (0 and 2 mM H_2SiO_3). There were four plants per pot. Letters belong to each cultivar separately and data are shown as means \pm SE of three replicates.



(LSD_{0.01} for three way interaction= 5.32)

Figure 6.3 Shoot Si concentration (mg g⁻¹) of eight maize cultivars supplied with different rates of salinity (0 and 50 mM NaCl) and Si (0 and 2 mM H₂SiO₃). There were four plants per pot. Letters belong to each cultivar separately and data are shown as means ± SE of three replicates

6.4 Discussion

Salinity stress significantly reduces shoot fresh and dry weights in crops at early growth stages (Kafi and Rahimi, 2011). Symptoms of salt stress started appearing two weeks after sowing particularly at highest level of NaCl salinity, so at early vegetative stage, there was a reduction in number of tillers, leaf size and plants weight (Table 6.1). Measurement of shoot growth is an effective criterion to screen cereal genotypes for salt tolerance at early growth stages. Elevated rate of salinity @ 80 and 160 mM has been reported to reduce shoot growth more severely than root growth of wheat seedlings (El-Hendawy, 2011). In wheat and barley, the seedling or early vegetative growth stage is known to be more sensitive to salt stress compared with later growth stages (Bhutta and Hanif, 2010; Khayatnezhad and Gholamin, 2010). Maize, a glycophyte crop was also reported as sensitive at early growth stages but tolerant at later stages (Khatoon et al., 2010). Akram et al. (2010) also mentioned the reduction of maize biomass yield in the presence of salinity. It might cause the inhibition of cell enlargement owing to less turgor pressure in higher levels of salt stress causing a reduction in shoot growth. It is postulated that higher salinity levels could enhance the inhibitors production like abscisic acid and hampered the plant growth promoter's synthesis like cytokinins (Munns and Tester 2008); as salinity stress exhibited marked reduction in dry weight (Table 6.1) and shoot K concentration (Figure 6.2) of cultivar EV 1089. Salinity stress significantly increased Shoot Na concentration with a marked reduction in shoot K concentration (Figure 6.1, 6.2), which might be due to specific ion toxicity (Munns et al., 2006). High requirement of K^+ was reported for cell expansion, osmoregulation, stomatal opening and carbon dioxide (CO_2) supply for photosynthesis (Munns and Tester, 2008).

Silicon application to a salt stressed maize plant increased the shoot growth and its biomass yield (Table 6.1). It is well documented that exogenously applied Si significantly ($p < 0.05$) enhances plant biomass under saline regimes (Moussa, 2006; Parveen and Ashraf, 2010). This increase in biomass might be due to higher mineral nutrient concentration like iron, phosphorus, calcium and magnesium in Si treated plants under salt stress (Mateos-Naranjo et al., 2013).

When Si was applied to saline growth medium, it not only decreased the Na^+ concentration but also enhanced the K^+ concentration in maize shoots (Figure 6.2, 6.3). Reduction in Na uptake and increase in K concentration has also been reported in wheat (Tahir et al., 2006; 2012) and in barley (Tuna et al., 2008). Silicon application also increases cell-wall Na^+ binding in wheat

(Saqib et al., 2008). In the present study, some maize cultivars survived up to 50 mM NaCl of soil salinity and, therefore, these cultivars can be classified as moderately salt tolerant. However, these cultivars can be categorized on their response to applied salinity like, Syngenta-8441 and ICI Hybrid showed excellent growth and shoot K concentration. Cultivar EV-1089 showed poor growth as compared to other cultivars under salinity however it responded effectively to apply Si and dry matter was ranged from 0.92-1.91 g (Table 6.2).

There was a decrease in shoot Si concentration by salinity stress (Figure 3). Mateos-Naranjo et al. (2013) reported that higher salinity levels suppress the Si concentration in both root and shoots in *Spartina densiflora*.

6.5 Conclusion

Salinity stress significantly reduces the plant growth by increasing Na concentration in different cultivars; however the reduction was variable among cultivars. Shoot K and Si concentration were significantly reduced in salt sensitive cultivar (EV 1089) as compared to salt tolerant (Syngenta 8441). Silicon treated maize plants perform better than non treated plants in saline conditions. Silicon alleviated the toxic effect of Na and increased the K concentration in maize shoot. Growth of cultivar Syngenta 8441 was least affected by salt stress; while cultivar EV 1089 was regarded as salt sensitive; these results were similar as shown in previous germination studies.

Silicon induced physiological and biochemical mechanisms of salinity tolerance in maize

Abstract

Food security is now becoming a global issue due to severe degradation of land by soil salinity. It hampers the growth of maize cultivars by osmotic and oxidative stresses. The main rationale of this dissertation was to evaluate the Si mediated performance of defensive mechanism of salt stressed maize cultivars with detailed investigation of photosynthetic apparatus. Two independent experiments were carried out in Pakistan and Austria including two cultivars (Syngenta 8441 and EV 1089) in hydroponic and sand culture solutions, respectively. Salinity stress significantly reduced the production of antioxidant enzymes and total phenolics directing to poor defensive mechanism of both maize cultivars against oxidative stress. Similarly, inefficient working of photosynthetic apparatus including photochemical efficiency of photosystem II and negative correlation of shoot sodium concentration with shoot potassium and dry matter leads to osmotic stress on maize cultivars. Silicon addition alleviated both osmotic and oxidative stress on maize crop by improving the contents of defensive machinery and water use efficiency. It increased root and shoot potassium concentration and dry weight of whole maize plants. It enhances maximum quantum yield of primary photochemistry which leads to smooth electron transport chain; ultimately lowers production of reactive oxygen species in chloroplast or mitochondria under salt stress. Therefore, this study implies that silicon treated maize plants have better chance to survive under salt stress conditions as their physiological and biochemical apparatus is working far better than non-silicon treated plants.

7.1 Introduction

Soil salinity generally prevails in arid to semi-arid regions around the globe. More than 6.3 m ha land in Pakistan is affected by salt stress including 2.67 m ha in Punjab (Alam et al., 2000). It generally causes physiological drought with least availability of water and ionic toxicity (Tester and Davenport, 2003). As a result, it causes reduction in dry matter of plant, low chlorophyll

content (Nasim et al., 2008) and inhibition of photosynthesis. Plants adjust osmotically to grow in saline conditions and also maintain positive turgor pressure. Similarly, solute concentration inside the cells always remains higher than external solution; but how much higher is still unknown for glycophytes because turgor pressure is rarely measured directly (Flowers et al., 2015). In cereals, Na^+ and Cl^- are the main source of disturbing osmotic potential inside the plants, so exogenous application of mineral nutrient is necessary to combat these toxic ions.

Silicon availability in the soil has never been questionable; as wide range of plants can uptake Si from soil solution freely. It is beneficial nutrient because under normal conditions, plants can complete their growth and development without Si (Epstein, 2000). In recent years, a lot of investigations and research has been conducted to check the role of Si in biotic and abiotic stresses (Raven, 2001; Liang et al., 2007; Mateos-Naranjo, 2013) with a special emphasis on agronomic crops like wheat, rice and barley (Yeo et al., 1999; Liang et al., 2007; Tuna et al., 2008). These species are mostly considered sensitive to the applied stress and Si application proved to be helpful to combat any kind of these stress i.e metal toxicity, drought or salt stress (Ma, 2004). Increasing the availability of Si in the growth medium can reduce salinity stress in plants by altering soil and plant factors (Kafi and Rahimi, 2011), but specific mechanisms are still debatable. Liang et al. (2007) reported that silicon uptake in salt stressed plant increases root activity for nutrient uptake, inhibits transpiration which reduces osmotic stress. It also increases the activity of ATPase & PPase in plasma membrane which ultimately increases K and decreases Na uptake (Tuna et al., 2008).

Salt stress also causes oxidative stress, on plants which leads to the production of reactive oxygen species. Reactive oxygen species (ROS) are highly reactive species produced inside the plant cell by more than one ways like; free oxygen radical (O_2^\bullet , OH^\bullet , HO_2^\bullet , RO^\bullet , alkoxy radicals, superoxide radicals; hydroxyl radical; perhydroxy radical) and non-radical (molecular) forms (H_2O_2 , $^1\text{O}_2$, hydrogen peroxide and singlet oxygen). In peroxisome, H_2O_2 and O_2^\bullet are produced at small electron transport chain (ETC). The major sites for the generation of $^1\text{O}_2$ and $\text{O}_2^{\bullet\bullet}$ in cytoplasm are Photosystem I and II (Del Rio et al., 2006). Similarly, complex I, ubiquinone and complex III of ETC in mitochondria are the major sites for the production of $\text{O}_2^{\bullet\bullet}$ (Navrot et al., 2007). The overproduction of ROS in plants is toxic and cause damage to the subcellular organelles like proteins, carbohydrates, lipids and DNA which ultimately leads to the

cell death. There are different mechanisms adapted by plants to cope up ROS and maintain their healthy growth in saline environment like production of antioxidant enzymes. Superoxide radical O_2^{\bullet} is dismutated by superoxide dismutase (SOD) which is reduced to H_2O_2 and O_2 (Prashanth, 2008; Wang, 2010). Then this H_2O_2 is converted into H_2O by catalases (CAT), ascorbate peroxidase (APX) and glutathione peroxidase (GPX) (Lu, 2007; Tseng, 2007). Silicon also stimulates the leaf superoxide dismutase activity and increased the activity of root H^+ -ATPase in the cell membrane of cucumber (Zhu et al., 2004). There is also low molecular weight, non-enzymatic antioxidants like α -tocopherol (to prevent lipid peroxidation), ascorbic acids and carotinoids present inside the cereals to combat salt stress.

In barley, lipid peroxidation which is caused by salt stress, was suppressed by Si application through decreased effect of malondialdehyde (compound which causes lipid peroxidation) and increased effect of α -tocopherol. This all discussion revealed that Si improves plasma membrane integrity (Tahir et al., 2012), structure and functions by manipulating the peroxidation (stress dependent process) of membrane lipids (Liang et al., 2003). However, the information on the role of Si in alleviating the salinity induced harmful effects on maize crop is not much explored. Moreover, genotypic variation among maize cultivars in response to applied salinity and silicon has not been studied in detail. In preliminary experiments we categorized maize cultivars according to their salinity tolerance. Two cultivars with contrasting salinity tolerance were selected to identify possible salinity tolerance mechanisms induced by Si application. Therefore, the main idea of present experiment was to evaluate the performance of defensive mechanism of salt stressed maize cultivars with Si application partially of activity of antioxidant enzymes and performance of photosystem II.

7.2 Materials and methods

7.2.2 Study 1

A hydroponic study was conducted in the wire-house, Institute of Soil and Environmental Sciences (ISES), University of Agriculture Faisalabad, Pakistan. Seeds of two selected (salt sensitive and tolerant) maize cultivars were sterilized in sodium hypochlorite solution (0.1%) for 5 min. To raise the nursery, well washed river sand was taken in plastic trays and seeds were sown after rinsing with distilled water. After 10 days, the uniform sized (with two expanded

leaves) seedlings were transferred to small pots containing continuously aerated 128 L nutrient solution, which contains macro-elements ($\text{Ca}(\text{NO}_3)_2$, 2mmol L⁻¹; KH_2PO_4 , 0.2 mmol L⁻¹; K_2SO_4 , 4 mmol L⁻¹; $(\text{NH}_4)_2\text{SO}_4$, 0.5 mmol L⁻¹; MgSO_4 , 1 mmol L⁻¹;) and micro-elements (H_3BO_3 , 25 $\mu\text{mol L}^{-1}$; MnSO_4 , 2 $\mu\text{mol L}^{-1}$; ZnSO_4 , 2 $\mu\text{mol L}^{-1}$; $(\text{NH}_4)_2\text{MoO}_7$, 0.5 $\mu\text{mol L}^{-1}$; CuSO_4 , 1 $\mu\text{mol L}^{-1}$; Fe-EDTA, 0.1 mmol L⁻¹). There were two levels of Si (0 and 2mM H_2SiO_3) and two levels of salinity stress (0 and 60 mM NaCl). The Si and NaCl treatments were applied after five days of transplantation in two equal splits. Each treatment was replicated four times. Pots were randomized accordingly.

The pH of the treatment solution was maintained at 6.5 using 0.01 N KOH or 0.01 N HCl. The treatment solutions were well aerated and changed weekly. Five gas exchange parameters like carbon dioxide assimilation rate (A), transpiration rate (E), stomatal conductance (gs), Internal carbon dioxide concentration (Ci) and water use efficiency (WUE) were recorded after 30 days of transplantation with CIRAS-3. After 40 days, plants/replicate of each treatment were harvested and rinsed with distilled water and fresh and dry weights were recorded.

7.2.2.1 Chlorophyll concentration

Chlorophyll extraction was calculated on fresh, fully expanded leaf material; a 1 g leaf sample was ground in 90% acetone using a pestle and mortar. The absorbance was measured with a UV/visible spectrophotometer (Shimadzu, UV-1201, Kyoto, Japan) and chlorophyll concentrations were calculated using the equation proposed by Strain and Svec (1966).

$$\text{Chl.a (mg ml}^{-1}\text{)} = 11.64 \times (\text{A663}) - 2.16 * (\text{A645})$$

$$\text{Chl.b (mg ml}^{-1}\text{)} = 20.97 \times (\text{A645}) - 3.94 * (\text{A663})$$

(A663) and (A645) represent absorbance values read at 663 and 645 nm wavelengths, respectively.

7.2.2.2 Total phenolics in shoots and roots

Total phenolics in the leaf water extracts were determined colorematically following the method described by Singleton et al. (1999). Two milliliters of the reaction mixture prepared by adding

20 μL sample extract, 1580 μL DI water, 100 μL Folin ciocalteu's reagent (0.25 N) and 300 μL Na_2CO_3 (1 N). The mixture was allowed to stand for 2 h in the dark at room temperature.

Absorbance of the sample reaction mixture and gallic acid standards was measured at 760 nm on spectrophotometer (Shimadzu, UV-1201, Kyoto, Japan). The concentration of the total phenolics was expressed in $\mu\text{g g}^{-1}$.

7.2.2.3 Total protein content

Protein content in the green leaves of stressed and non-stressed plants was determined following Bardford (1976) method. For analysis 200 μL of sample extract was taken into a test tube with the addition of 1800 μL DI water. Then 2 mL Bardford reagent was added and the mixture was incubated at room temperature for 10-20 minutes. After incubation, absorbance was recorded at 595 nm wavelength using spectrophotometer (Shimadzu, UV-1201, Kyoto, Japan). Concentration of protein (mg g^{-1} fresh weight) was calculated by standard curve using different concentrations of bovine serum albumin (BSA).

7.2.2.4 Crude leaf extract for antioxidant enzyme assays

Fresh leaf tissue was collected from stressed and well-watered plants of both transgenic and control lines. Approximately 200 mg of leaf tissue was weighed and ground to a fine powder using a precooled mortar and pestle. The exact weight of each powdered sample was determined before it was thoroughly homogenized in 1.2 mL of 0.2 M potassium phosphate buffer (pH 7.8 with 0.1 mM EDTA). The samples were centrifuged at $15,000\times g$ for 20 min at 4°C . The supernatant was removed, the pellet resuspended in 0.8 mL of the same buffer, and the suspension centrifuged for another 15 min at $15,000\times g$. The combined supernatants were stored on ice and used to determine different antioxidant enzyme activities.

7.2.2.5 Superoxide dismutase in leaves

Total SOD activity was assayed using a modified NBT method (Bayer et al., 1987). The 2 mL assay reaction mixture contained 50 mM phosphate buffer (pH 7.8) containing 2 mM EDTA, 9.9 mM L-methionine, 55 μM NBT, and 0.025% Triton-X100. Forty microliters of diluted ($2\times$) sample and 20 μL of 1 mM riboflavin were added and the reaction was initiated by illuminating the samples under a 15 W fluorescent tube. During the 10-min exposure, the test tubes were

placed in a box lined with aluminum foil. The box with the test tubes was placed on a slowly oscillating platform at a distance of approximately 12 cm from the light source. Duplicate tubes with the same reaction mixture were kept in the dark and used as blanks. Absorbance of the samples was measured on UV/visible spectrophotometer (Shimadzu, UV-1201, Kyoto, Japan) immediately after the reaction was stopped at 560 nm. The enzyme activity (unit mg protien⁻¹) of a sample was determined from a standard curve obtained by using pure SOD.

7.2.2.6 Ascorbate peroxidase in leaves

APX activity was assayed using a modified method of (Nakano and Asada, 1981). APX activity was determined from the decrease in absorbance at 290 nm due to oxidation of ascorbate in the reaction. The 1 mL assay mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.5 mM H₂O₂, and 10 µL of crude leaf extract. H₂O₂ was added last to initiate the reaction, and the decrease in absorbance was recorded for 3 min on UV/visible spectrophotometer (Shimadzu, UV-1201, Kyoto, Japan). The extinction coefficient of 2.8 mM⁻¹ cm⁻¹ for reduced ascorbate was used in calculating the enzyme activity that was expressed in terms of mM Ascorbate min⁻¹ mg⁻¹ protein.

7.2.2.7 Catalase in leaves

Reaction mixture (3 mL) containing 2 mL enzyme extract, (diluted 200 times with 50 mM, pH 7.0 potassium phosphate buffer) and 1 mL H₂O₂ (10 mM) was measured by decrease in absorbance at 240 nm on UV/visible spectrophotometer (Shimadzu, UV-1201, Kyoto, Japan) due to H₂O₂ extinction. The activity was calculated in terms of mM H₂O₂ min⁻¹ mg⁻¹ protein at 25 ± 2 °C (Cakmak and Marschner, 1992).

7.2.3 Study 2

A sand culture experiment was conducted in the greenhouse of Health and Environment Department, Austrian Institute of Technology Gmbh in Tulln (Austria). Seeds of two selected (salt sensitive and tolerant) maize cultivars were sterilized in sodium hypochlorite solution (0.1%) for 5 min. To raise the nursery, well washed river sand was taken in plastic trays and seeds were sown after rinsing with distilled water. After 10 days, the uniform sized (with two

expanded leaves) seedlings were transferred to small pots containing 5 Kg sand and placed each pot separately in a tub containing 64 L nutrient solution, which contains macro-elements (Ca (NO₃)₂, 2mmol L⁻¹; KH₂ PO₄, 0.2 mmol L⁻¹; K₂ SO₄, 4 mmol L⁻¹; (NH₄)₂ SO₄, 0.5 mmol L⁻¹; Mg SO₄, 1 mmol L⁻¹;) and micro-elements (H₃BO₃, 25 µmol L⁻¹; Mn SO₄, 2 µmol L⁻¹; Zn SO₄, 2 µmol L⁻¹; (NH₄)₂MoO₇, 0.5 µmol L⁻¹; Cu SO₄, 1 µmol L⁻¹; Fe-EDTA, 0.1 mmol L⁻¹). There were two levels of Si (0 and 2mM H₂SiO₃) and two levels of salinity stress (0 and 60 mM NaCl). The Si and NaCl treatments were applied after five days of transplantation in two equal splits. Each treatment was replicated four times. Pots were randomized accordingly.

The pH of the treatment solution was maintained at 6.5 using 0.01 N KOH or 0.01 N HCl. The treatment solutions were well aerated and changed weekly.

7.2.3.1 Chlorophyll content

Chlorophyll content was measured using SPAD-502 meter (Konica-Minolta, Japan). Readings were recorded with three repeats from each treatment.

7.2.3.2 Measurement of Gas exchange parameters

Gas exchange measurements were taken on randomly selected, fully expanded penultimate leaves using an infrared gas analyzer in an open system after 30 days of transplantation with LICOR (LI-6400, LI-COR Inc., Neb., USA). Five gas exchange parameters like carbon dioxide assimilation rate (A), transpiration rate (E), stomatal conductance (gs), water use efficiency (WUE) (Ahmad *et al.*, 2013), and intrinsic water use efficiency (Fischer *et al.*, 1998) were recorded at ambient CO₂ concentration of 390 ppm, temperature of 26 °C, 55±5% relative humidity and a photon flux density of 1002 mmol m⁻² s⁻¹.

7.2.3.3 Measurement of chlorophyll fluorescence

Chlorophyll fluorescence was measured in random, fully developed penultimate leaves (n ¼ 10, one per plant and three extra taken randomly) using a portable modulated fluorometer (Mini-PAM, Heinz Walz, Germany) after 30 days of treatment. Light- and dark-adapted fluorescence parameters were measured at midday (1600 mmol m⁻² s⁻¹). Plants were dark-adapted for 20 min, using manufacturers' leaf clips. The minimal fluorescence level in the dark-adapted state (F₀) was measured using a modulated pulse (<0.05 mmol m⁻² s⁻¹ for 1.8 ms) which was too small to induce significant physiological changes in the plant. The data stored were an average taken over a 1.6 s period. Maximal fluorescence in this state (F_m) was measured after applying a saturating

actinic light pulse of $10,000 \text{ mmol m}^{-2} \text{ s}^{-1}$ for 0.8 s. The value of F_m was recorded as the highest average of two consecutive points. Values of the variable fluorescence ($F_v/4F_m - F_0$) and maximum quantum efficiency of PSII photochemistry (F_v/F_m) were calculated from F_0 and F_m . This ratio of variable to maximal fluorescence correlates with the number of functional PSII reaction centres, and dark-adapted values of F_v/F_m can be used to quantify photoinhibition (Maxwell, 2000).

After 40 days, plants/replicate of each treatment were harvested and rinsed with distilled water and blotted dry with tissue paper. The plant samples were divided into root and shoot, air-dried and then oven dried at 65°C in a forced air driven oven to a constant weight. Fresh and dry matter yield was taken and plant samples were ground.

7.2.3.4 Na and K concentration

Plant samples were tested for the Na^+ and K^+ concentration in both root and shoot. Finely ground plant samples (0.1 g) were digested in a di-acid ($\text{HNO}_3:\text{HClO}_4$) mixture (Jones and Case, 1990). The Na and K concentration in the digest was estimated by flame photometer (Jenway, PFP-7).

Statistical analysis

Data was statistically analyzed by *Microsoft Excel 2010*® (Microsoft Cooperation, USA) and *Statistix 8.1*® (Analytical Software, Tallahassee, USA). Significantly different treatment means were separated using least significant difference (LSD) test (Steel et al., 1997).

7.4 Results

7.4.1 Study 1

7.4.1.1 Plant biomass

Salinity stress significantly ($p < 0.05$) decreased dry matter yield of both cultivars, however reduction was variable among both cultivars and different tissues (Fig. 7.1). Maximum reduction was observed in roots and old leaves when plants were grown under salinity stress. The dry matter yield was higher in cultivar Syngenta 8441 relative to cultivar EV 1089. Maximum reduction in root dry matter was observed in old leaves cultivar EV 1089 (57%), while cultivar Syngenta 8441 showed minimum reduction (20%) in old leaves relative to control. Silicon

addition significantly decreased the reduction in plant dry matter yield compared to salinity treatment only. The reduction in dry matter yield in old leaves of cultivar EV 1089 was decreased from 57 to 8% in treatment where plants were grown with Si under salinity stress.

7.4.1.2 Photosynthetic parameters and pigments

CO₂ assimilation rate (*A*) was significantly ($p<0.05$) influenced by the interactive effect of salinity, Si and cultivars (Figure 7.2). There was no statistical difference for (*A*) found among both cultivars at control. However, salinity stress significantly ($p<0.05$) decreased *A* of both cultivars; however reduction was variable among both cultivars. Silicon addition decreased the reduction in *A* compared to salinity treatment only. The reduction in *A* of cultivar EV 1089 was decreased from 41 to 33% in treatment where plants were grown with Si under salinity stress.

The absolute value of transpiration rate (*E*) was higher in cultivar Syngenta 8441 relative to cultivar EV 1089 under control conditions (Figure 7.2). Salinity stress significantly ($p<0.05$) decreased *E* of cultivar Syngenta 8441 but no significant difference was found among cultivar EV 1089 with applied stress. Silicon application had no significant influence on *E* in both cultivars at treatment where plants were grown with Si under salinity stress.

Stomatal conductance (*g_s*) of maize cultivars significantly ($p<0.05$) influenced by the interactive effect of salinity, Si and cultivars (Figure 7.4). The absolute value of *g_s* was higher in cultivar Syngenta 8441 relative to cultivar EV 1089 under control conditions. Salinity stress significantly ($p<0.05$) decreased *g_s* of both cultivars, however reduction was variable among both cultivars. Silicon addition decreased the reduction in *g_s* compared to salinity treatment only. The reduction in *g_s* of cultivar Syngenta 8441 was decreased from 45% to 23% in treatment where plants were grown with Si under salinity stress. However, there was no influence of salinity stress observed on internal CO₂ concentration (*C_i*) among both maize cultivars (Figure 7.3).

Salinity stress had a negative impact on pigments concentration (Chl. a and Chl. b) in both cultivars. There was a significant ($p<0.05$) decrease (60 %) in Chl. a observed in cultivar EV1089 with applied salinity stress (Figure 7.4); however cultivar Syngenta 8441 showed minimum reduction (40%) compared to control. Silicon application increased the Chl. a

concentration relative to saline treatment only. Chlorophyll a concentration of cultivar EV1089 was increased from 60 to 31% in treatment where plants were grown with Si under salinity stress compared to salinity treatment only.

Similarly, salinity stress significantly ($p<0.05$) decreased chl. b concentration of both cultivars (Figure 7.4); however reduction was variable among both cultivars. Silicon addition decreased the reduction in chl. b concentration compared to salinity treatment only. Chlorophyll b concentration in cultivar Syngenta 8441 increased 6 folds in treatment where plants were grown with Si under salinity stress relative to saline treatment only.

7.4.1.3 Total phenolics contents

Salt stress did not significantly ($p<0.05$) affect total phenolics contents in maize roots relative to control plants (Figure 7.5). Silicon application under salinity stress significantly ($p<0.05$) decreased (54%) the reduction in root total phenolics of cultivar EV 1089 compared to salinity treatment only. The reduction in root total phenolics of cultivar Syngenta 8441 was decreased from 18 to 2% in treatment where plants were grown with Si under salinity stress relative to control.

The absolute value of shoot total phenolics was higher in cultivar Syngenta 8441 relative to cultivar EV 1089 under control conditions (Figure 7.5). Salinity stress decreased shoot total phenolics contents among both cultivars; however reduction was variable among both cultivars. Silicon application enhanced the total phenolics contents relative to saline treatment, only. The reduction in shoot total phenolics of cultivar Syngenta 8441 was decreased from 54 to 29% in treatment where plants were grown with Si under salinity stress.

7.4.1.4 Antioxidant enzymes activity

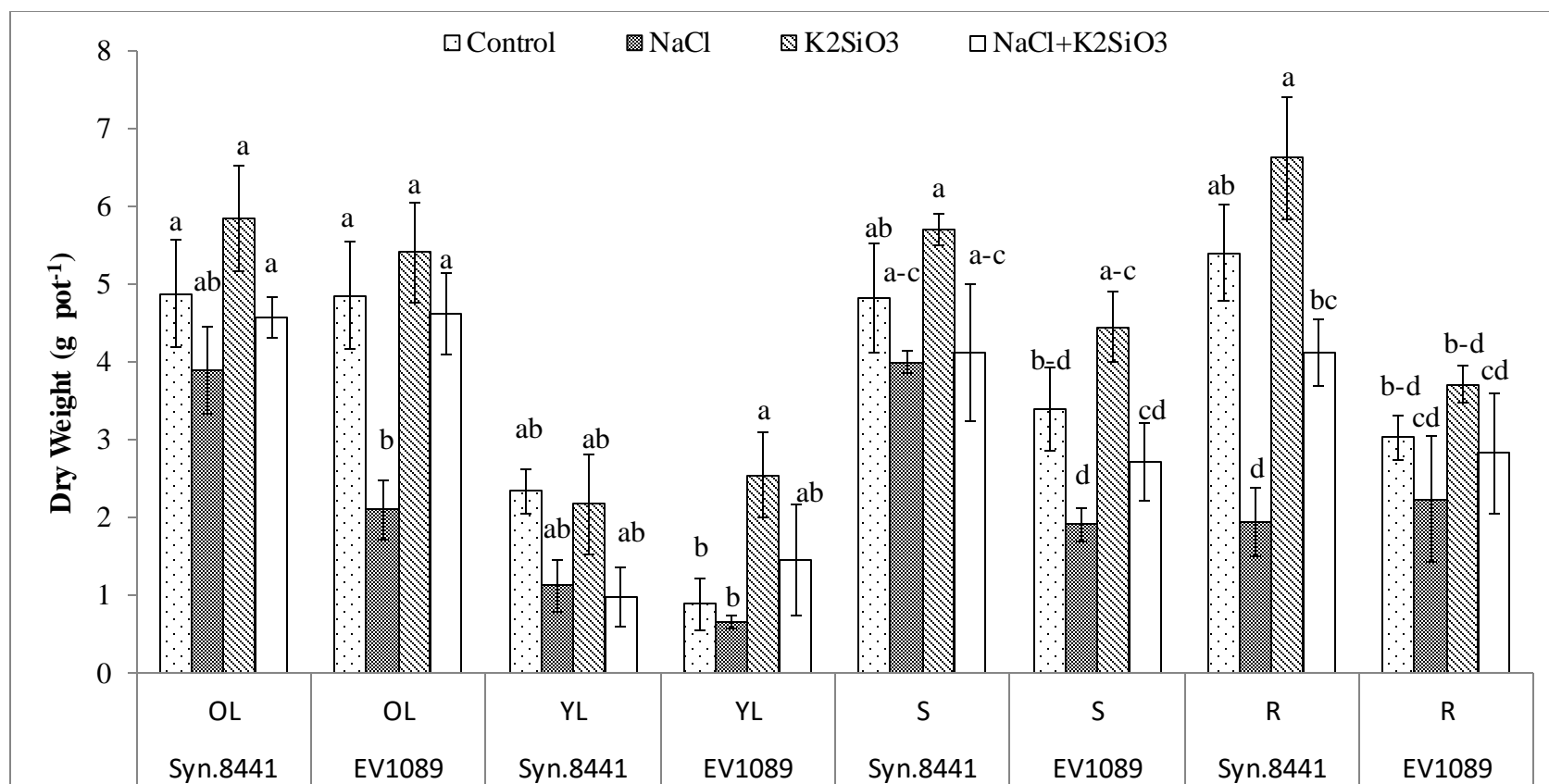
Both cultivars were not statistically different at control and saline treatment. Salinity stress decreased superoxide dismutase (SOD) contents of both cultivars; however reduction was variable among cultivars (Figure 7.6). Superoxide dismutase contents were increased with Si application compared to saline treatment only. There was two folds increase in SOD contents observed in cultivar EV1089 when plants were grown with Si under salinity stress relative to saline treatment only.

Ascorbate peroxidase (APX) contents of maize cultivars significantly ($p<0.05$) affected by the main effect of salinity, Si and cultivars (Figure 7.6). Both cultivars were not statistically different at control and saline treatment. Salinity stress significantly reduced (32%) APX contents in cultivar EV 1089 but Si application increased APX contents in both cultivars relative to saline treatment only. Cultivar EV 1089 showed 67% increase in APX contents when plants were grown with Si under salinity stress relative to saline treatment only.

The absolute value of catalase (CAT) was higher in cultivar Syngenta 8441 relative to cultivar EV 1089 under control conditions. Salinity stress reduced (CAT) contents in cultivar Syngenta 8441; however both cultivars are statistically similar under salt stress (Figure 7.7). Silicon application increased CAT contents in both cultivars relative to saline treatment only. There was 29% increase in CAT contents observed in cultivar EV 1089 when plants were grown with Si under salinity stress relative to saline treatment.

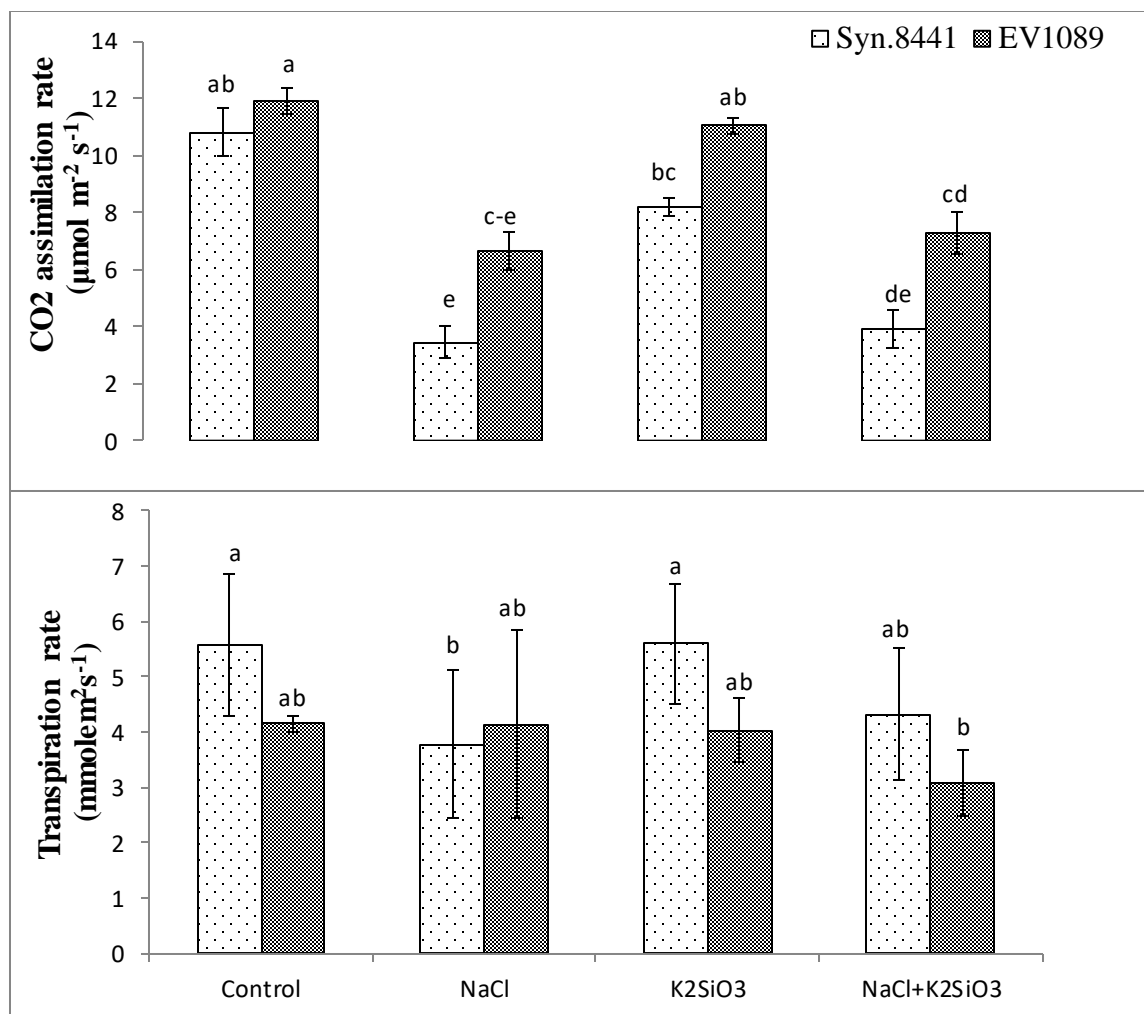
7.4.1.5 Total protein contents

Salinity stress significantly ($p<0.05$) decreased total protein contents of both cultivars (Figure 7.7); however reduction was variable among both cultivars. Maximum reduction in total protein contents was observed in cultivar EV 1089 (80%), while cultivar Syngenta 8441 showed minimum reduction (25%) relative to control. Silicon addition decreased the reduction in total protein contents compared to salinity treatment only. The reduction in total protein contents of cultivar EV 1089 was decreased from 80% to 61% because of Si application under salinity.



(LSD_{0.05}: interaction effect for OL= 2.0; for YL= 1.5; for S= 1.9; for R= 1.9)

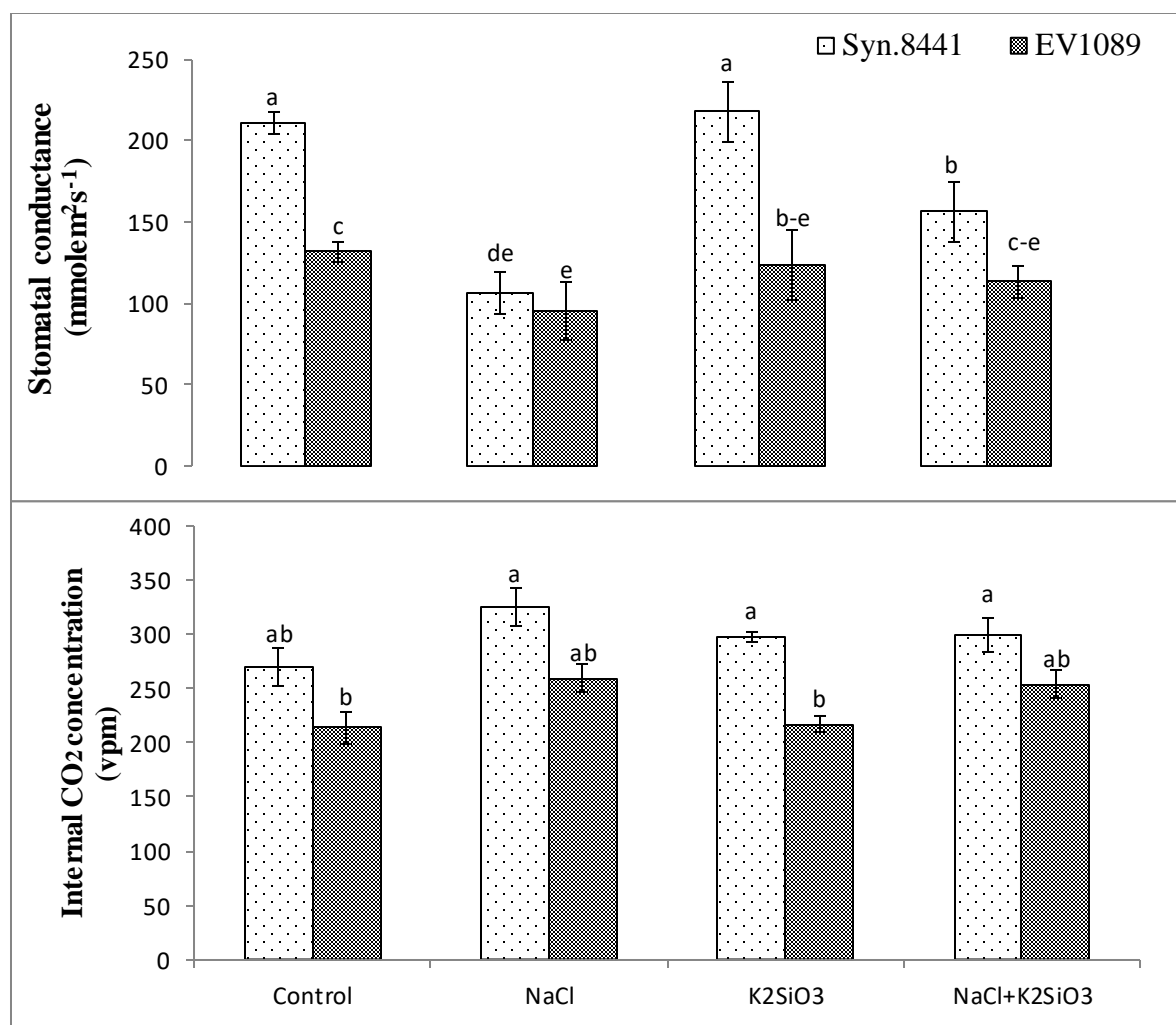
Figure 7.1 Dry weight (g pot⁻¹) of 40 days old maize cultivars influenced by the Si application under salt stress developed after plants established in nutrient solution in pots where bars sharing similar letters are statistically similar to each other at $p \leq 0.05$. Statistical analysis and letters belong to each plant tissue separately and values are means \pm S.E n=4 (OL= Old Leaf; YL= Young Leaf; S= Shoot; R= Root; Syn8441= Syngenta8441)



(LSD_{0.05}: interaction effect for $A = 3.4$)

(LSD_{0.05}: interaction effect for $E = 1.6$)

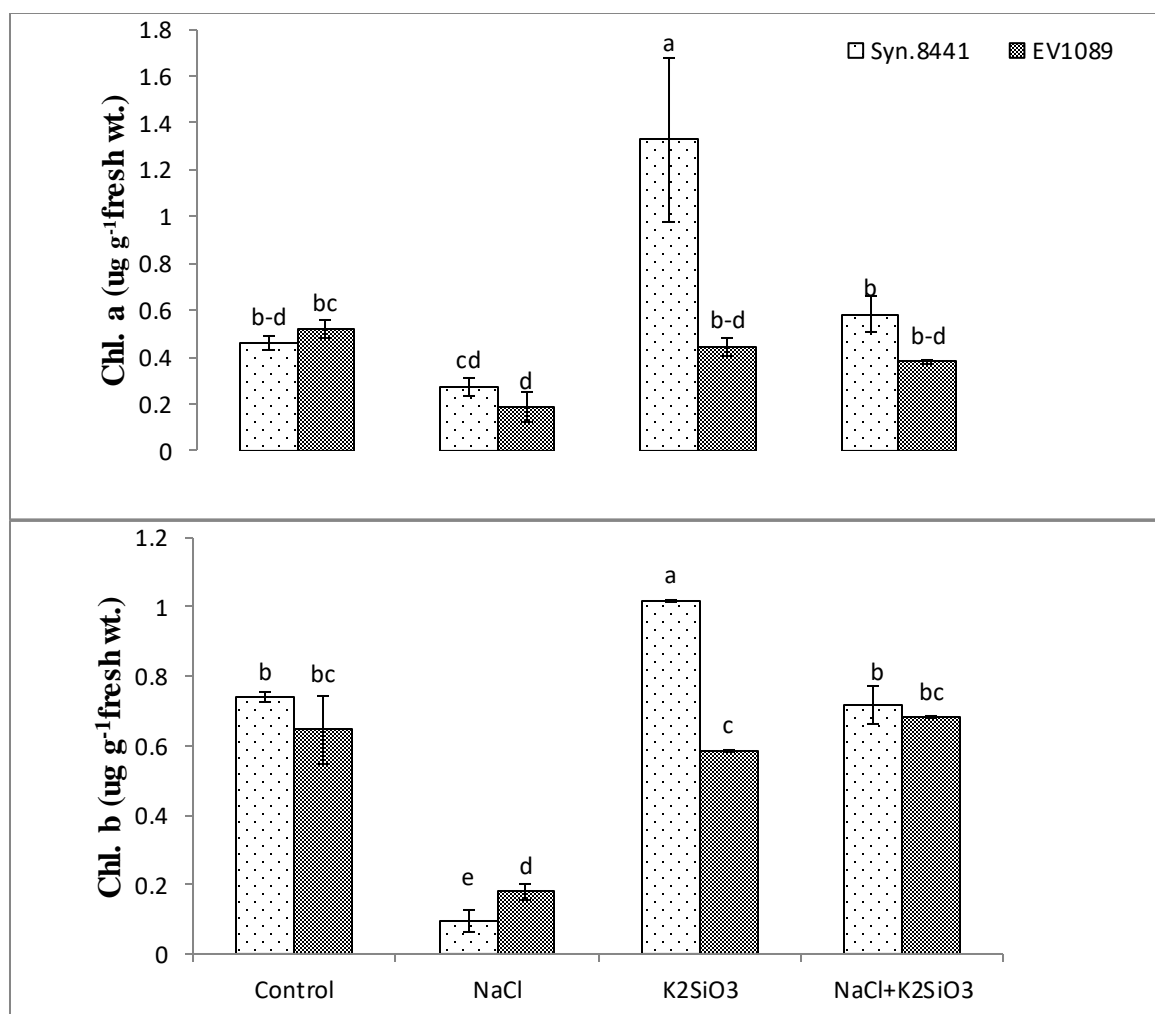
Figure 7.2 CO₂ assimilation rate (A) and Transpiration rate (E) of maize leaf influenced by the Si application under salt stress developed after plants established in nutrient solution in pots where bars sharing similar letters are statistically similar to each other at $p \leq 0.05$. Values are means \pm S.E $n=4$ (Syn8441= Syngenta8441)



(LSD_{0.05}: interaction effect for g_s = 22)

(LSD_{0.05}: interaction effect for C_i = 79.4)

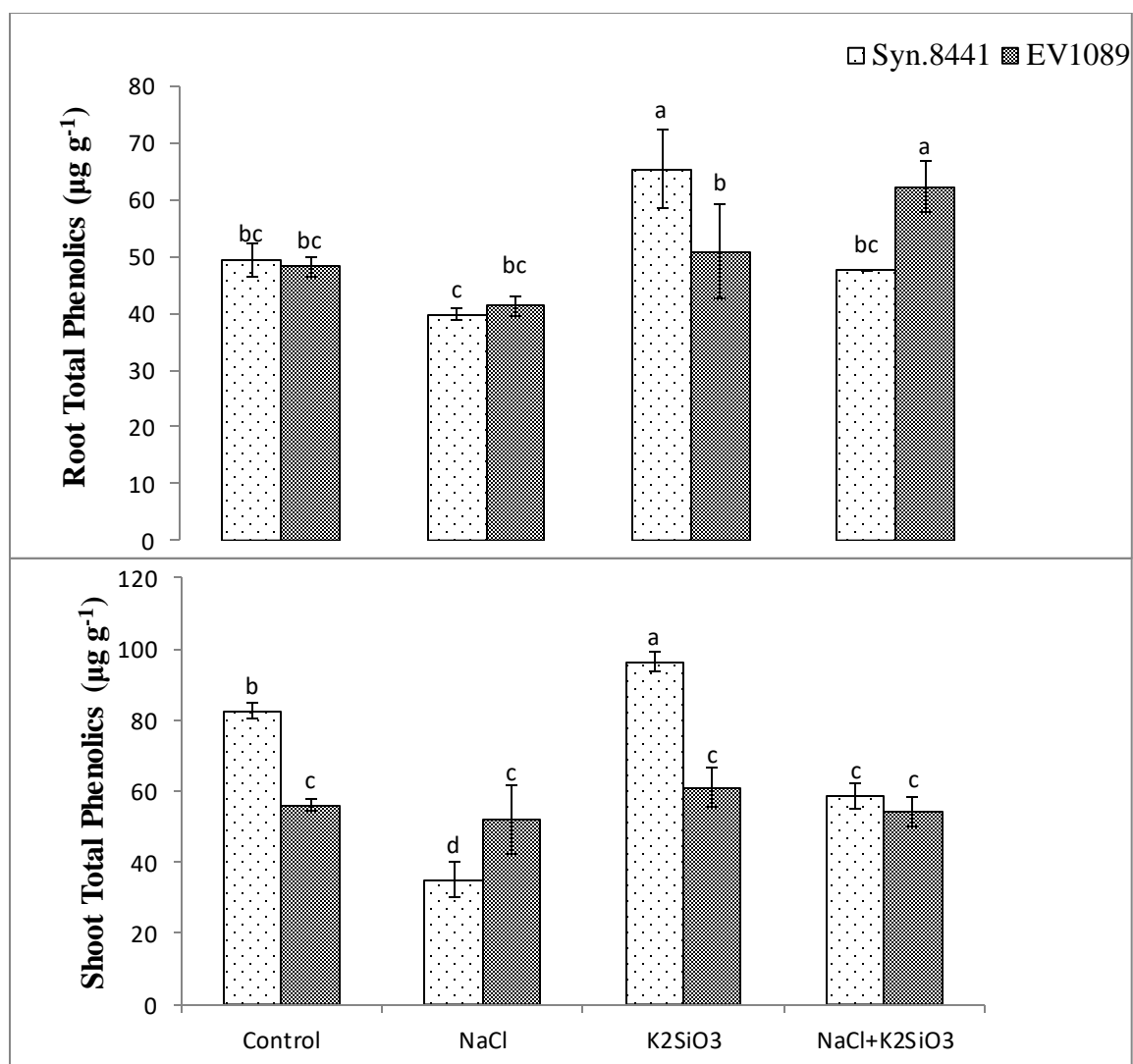
Figure 7.3 Stomatal conductance (g_s) and Internal CO₂ concentration (C_i) of maize leaf influenced by the Si application under salt stress developed after plants established in nutrient solution in pots where bars sharing similar letters are statistically similar to each other at $p \leq 0.05$. Values are means \pm S.E n=4 (Syn8441= Syngenta8441)



(LSD_{0.05}: interaction effect for Chl.a= 0.19)

(LSD_{0.05}: interaction effect for Chl. b= 0.23)

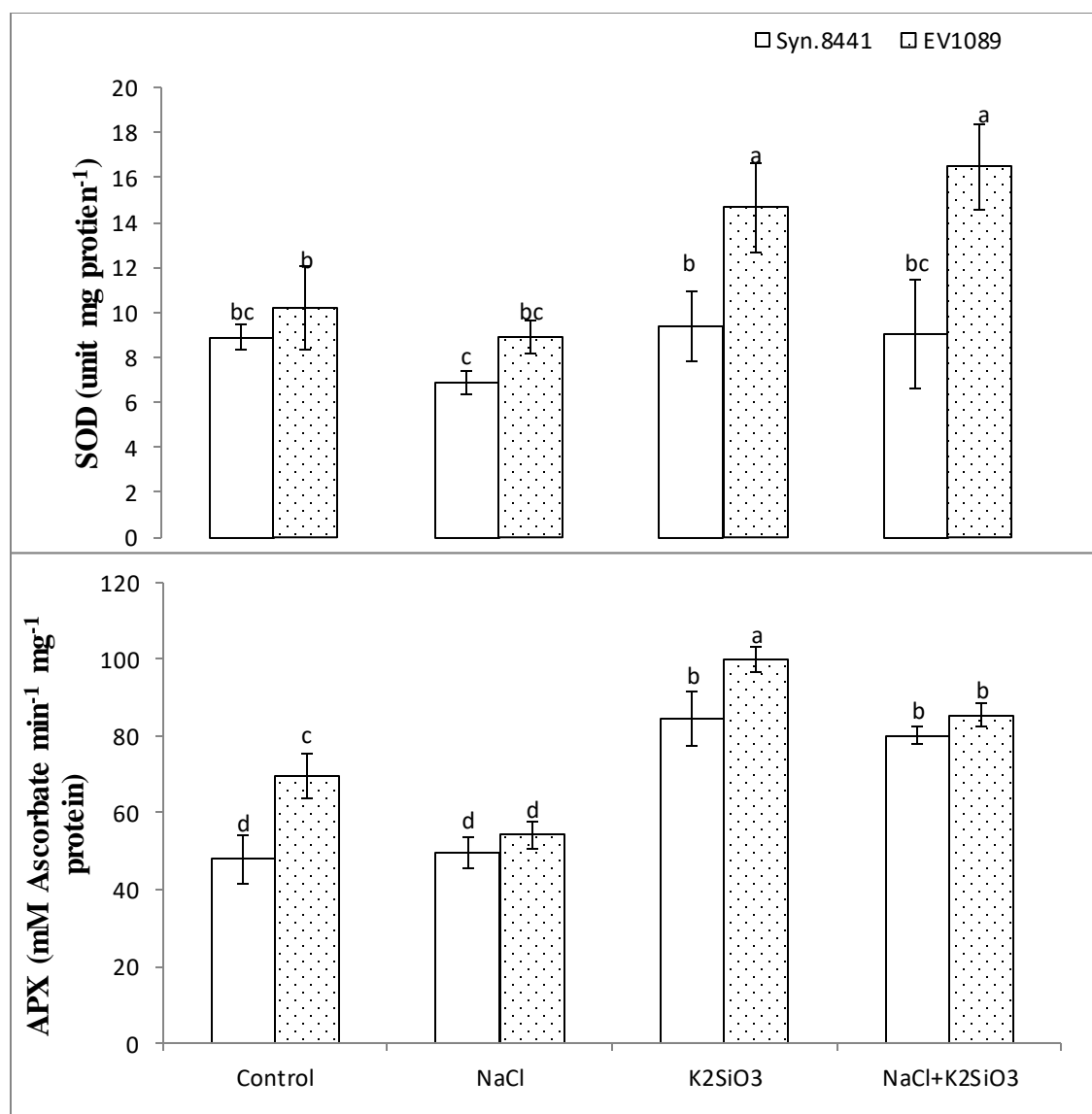
Figure 7.4 Chlorophyll a and Chlorophyll b of 40 days old maize cultivars influenced by the Si application under salt stress developed after plants established in nutrient solution in pots where bars sharing similar letters are statistically similar to each other at $p \leq 0.05$. Values are means \pm S.E n=4 (Syn8441= Syngenta8441)



(LSD_{0.05}: interaction effect for root total phenolics= 10.0)

(LSD_{0.05}: interaction effect for shoot total phenolics= 11.2)

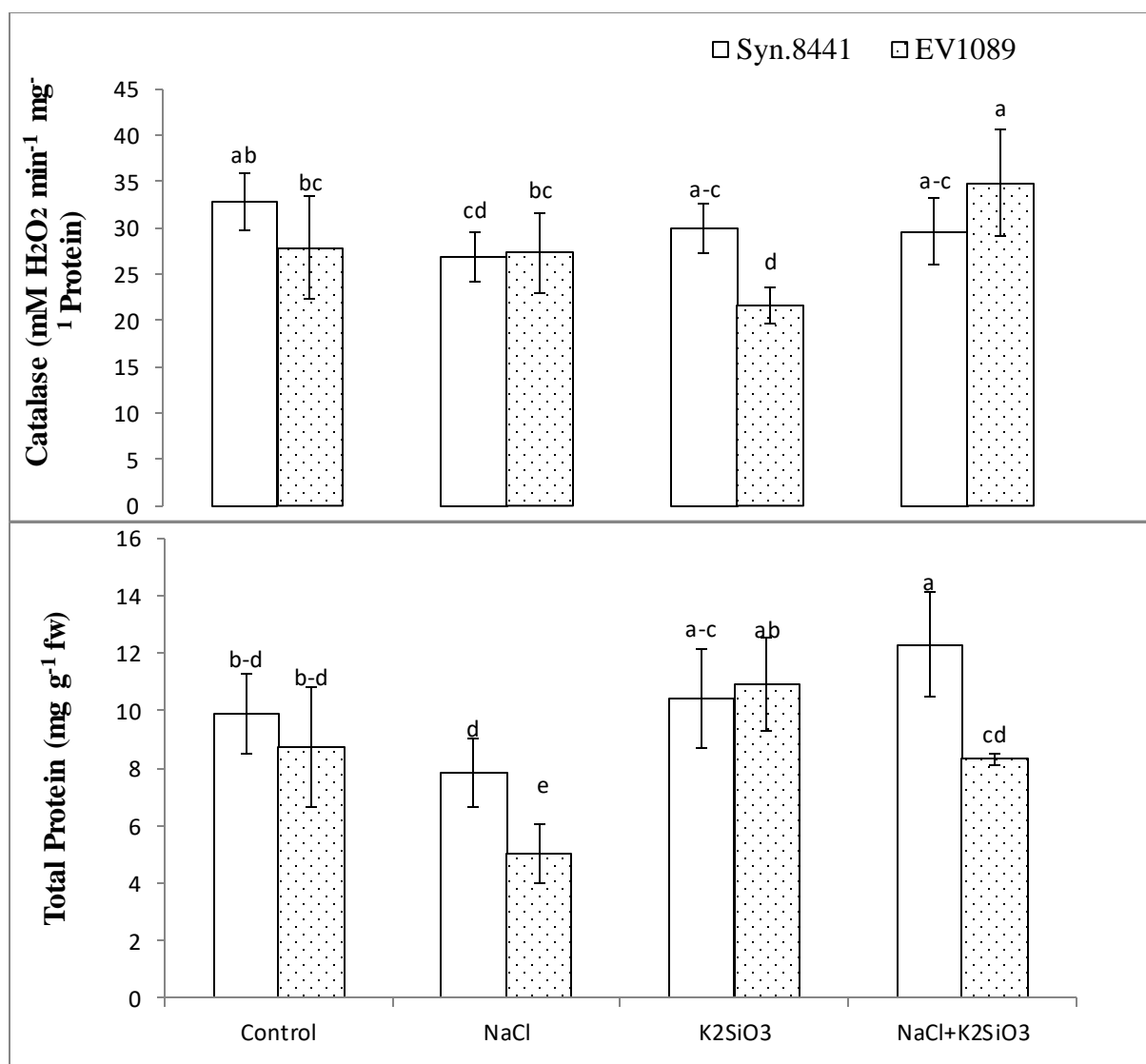
Figure 7.5 Root and shoot Total Phenolic contents of 40 days old maize cultivars influenced by the Si application under salt stress developed after plants established in nutrient solution in pots where bars sharing similar letters are statistically similar to each other at $p \leq 0.05$. Values are means \pm S.E n=4 (Syn8441= Syngenta8441)



(LSD_{0.05}: interaction effect for SOD= 2.34)

(LSD_{0.05}: interaction effect for APX= 6.84)

Figure 7.6 Superoxide dismutase (SOD) and Ascorbate peroxidase (APX) content of 40 days old maize cultivars influenced by the Si application under salt stress developed after plants established in nutrient solution in pots where bars sharing similar letters are statistically similar to each other at $p \leq 0.05$. Values are means \pm S.E n=4 (Syn8441= Syngenta8441)



(LSD_{0.05}: interaction effect for CAT= 4.24)

(LSD_{0.05}: interaction effect for total protein= 2.3)

Figure 7.7 Catalase (CAT) and Total Protein content of 40 days old maize cultivars influenced by the Si application under salt stress developed after plants established in nutrient solution in pots where bars sharing similar letters are statistically similar to each other at $p \leq 0.05$. Values are means \pm S.E n=4 (Syn8441= Syngenta8441)

7.4.2 STUDY 2

7.4.2.1 Photosynthetic parameters

The absolute value of transpiration rate (E) was higher in cultivar Syngenta 8441 relative to cultivar EV 1089 under control conditions (Figure 7.9). Photosynthetic rate (A) was significantly ($p<0.05$) influenced by the interactive effect of salinity, Si and cultivars. Salinity stress significantly ($p<0.05$) decreased A of both cultivars; however both cultivars were statistically similar at salt treatment. Silicon addition decreased the reduction in A compared to salinity treatment only. The reduction in A of cultivar EV1089 was decreased from 46 to 24% in treatment where plants were grown with silicon under salinity stress.

Salinity stress significantly ($p<0.05$) decreased transpiration rate (E) of both cultivars; however reduction was variable among cultivars (Figure 7.9). Higher reduction in E was observed in cultivar Syngenta8441 (68%), while cultivar EV1089 showed minimum reduction (31%) relative to control. In cultivar EV1089, Si addition increased the reduction in E compared to saline treatment only. The reduction in E of cultivar EV1089 was increased from 31 to 44% in treatment where plants were grown with silicon under salinity stress.

Stomatal conductance of CO_2 (g_s) in maize cultivars significantly ($p<0.05$) influenced by the interactive effect of salinity, Si and cultivars (Figure 7.9). Salinity stress significantly ($p<0.05$) decreased g_s of both cultivars, however reduction was variable among both cultivars. Higher reduction in g_s was observed in cultivar EV1089 (42%), while cultivar Syngenta8441 showed minimum reduction (39%) relative to control. Silicon addition decreased the reduction in g_s compared to salinity treatment only. The reduction in g_s of cultivar Syngenta8441 was decreased from 39 to 15% in treatment where plants were grown with silicon under salinity stress.

The absolute values were higher for WUE in cultivar Syngenta 8441 relative to cultivar EV1089 at control (Figure 7.10). Salinity stress decreased water use efficiency (WUE) of both cultivars; however reduction was variable among both cultivars. Silicon addition significantly decreased the reduction in WUE compared to saline treatment only. The reduction in WUE of cultivar Syngenta8441 was decreased from 55% to 3% in treatment where plants were grown with silicon under salinity stress.

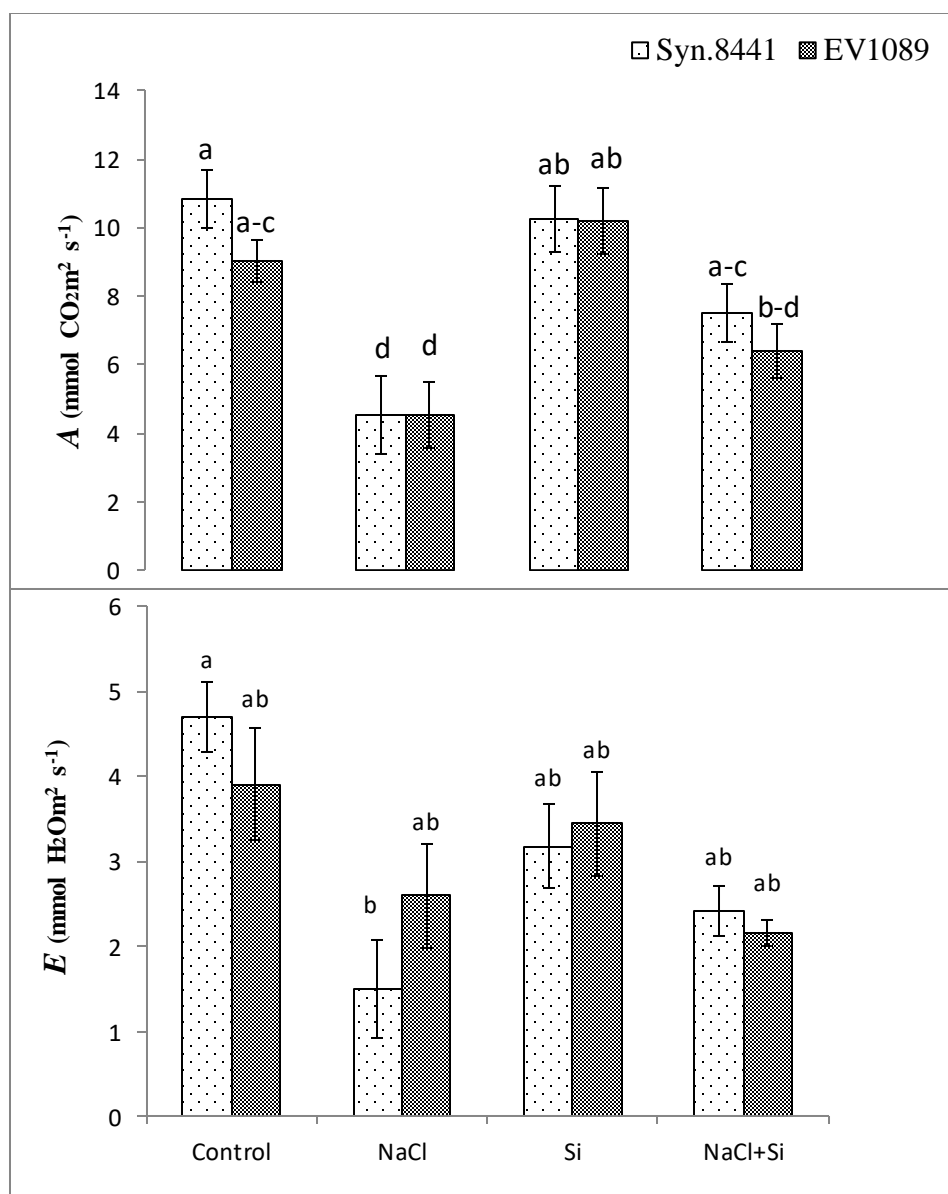
7.4.2.2 Chlorophyll fluorescence

Photochemical efficiency of photosystem II (Fv/Fm) in maize cultivars significantly ($p<0.05$) influenced by the interactive effect of salinity, Si and cultivars (Figure 7.11). The absolute values were higher for (Fv/Fm) in cultivar Syngenta 8441 relative to cultivar EV1089 at control. Salinity stress decreased Fv/Fm of both cultivars; however reduction was variable among both cultivars. Silicon addition decreased the reduction in Fv/Fm compared to saline treatment only. The reduction in Fv/Fm of cultivar Syngenta8441 was decreased from 59 to 11% in treatment where plants were grown with silicon under salinity stress.

7.4.2.3 Ionic concentration

Salinity stress increased the root and shoots Na concentrations of both cultivars; however reduction was variable among both cultivars (Table 7.1). Higher increase in shoot Na concentration (84%) was observed in cultivar EV1089, while cultivar Syngenta 8441 showed minimum root Na concentration (4%) relative to control. Silicon addition decreased the reduction in root and shoot Na concentration compared to saline treatment only; however Si application under salinity stress increased (42%) root Na concentration in cultivar EV 1089 relative to salinity stress.

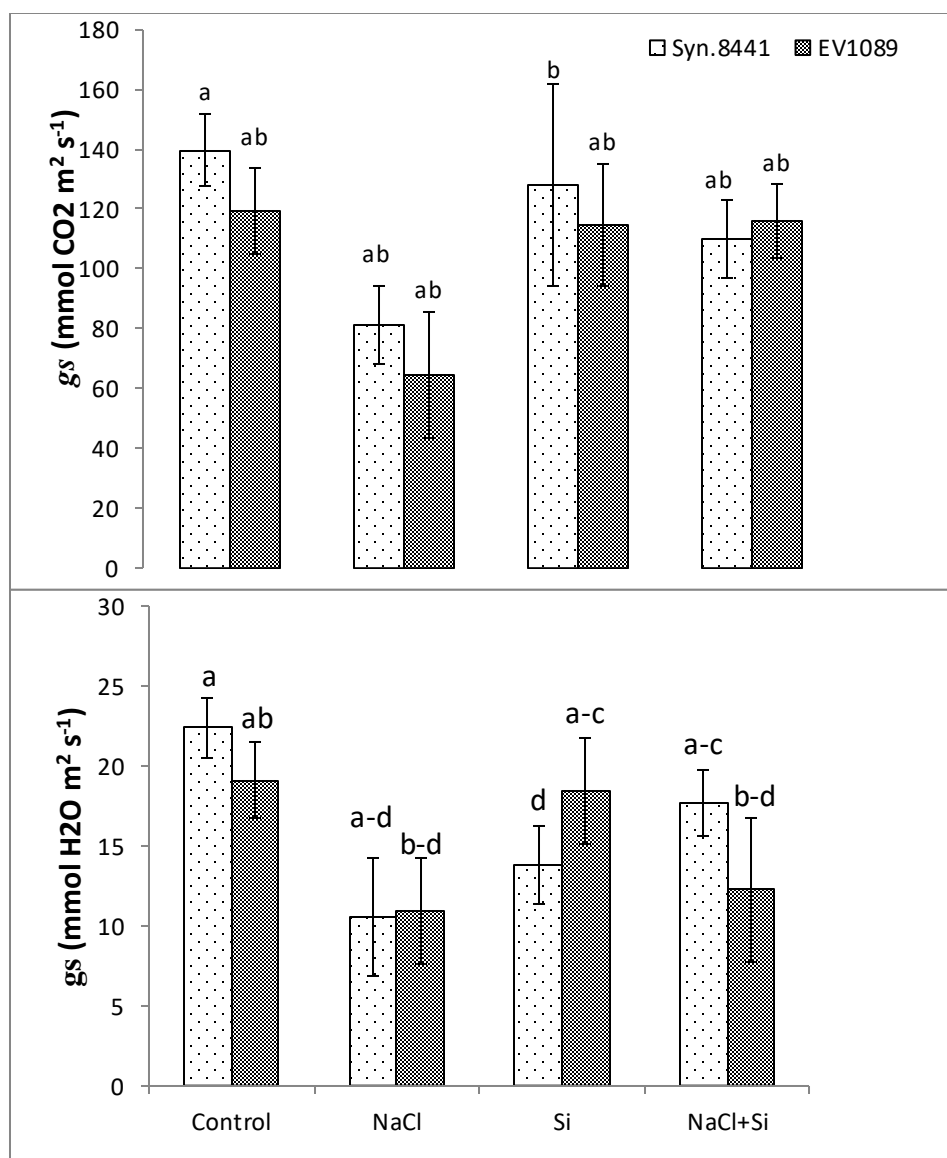
Salinity stress significantly ($p<0.05$) decreased root and shoot K concentrations of both cultivars; however reduction was variable among both cultivars. Higher reduction in shoot K concentration was observed in cultivar EV 1089 (60%), while cultivar Syngenta 8441 showed minimum reduction (59%) relative to control. Silicon addition decreased the reduction in root and shoot K concentrations compared to salinity treatment only. The reduction in shoot K concentration of cultivar EV 1089 was decreased from 60 to 7% in treatment where plants were grown with silicon under salinity stress.



LSD_{0.05} for A= 4.5

LSD_{0.05} for E= 2.2

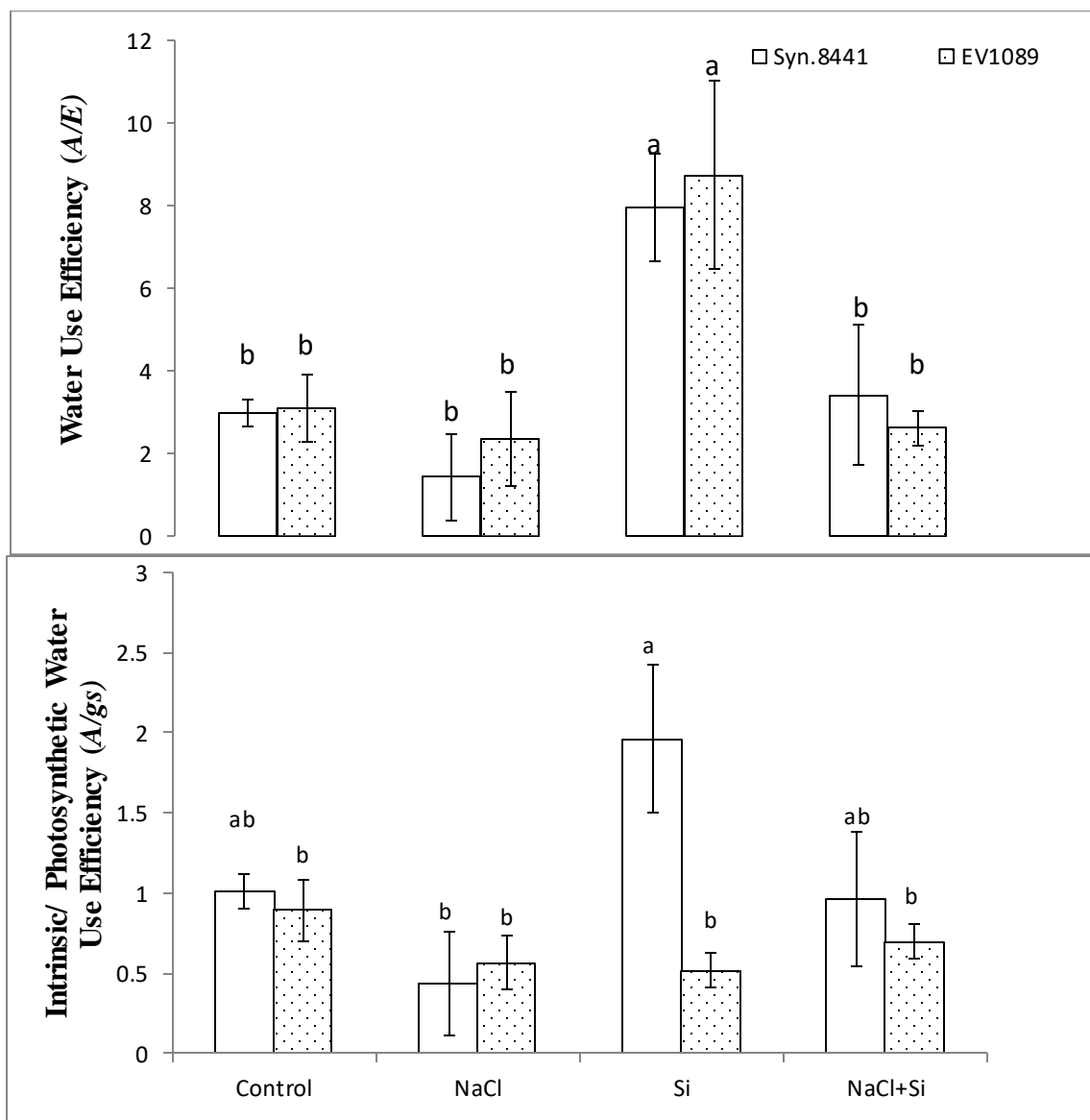
Figure 7.8 Photosynthetic rate (*A*) and transpiration rate (*E*) of maize cultivars with and without Si application under salt stress. Bars sharing similar letters are statistically similar to each other at $p \leq 0.05$. Letters belong to each parameter separately and values are means \pm S.E n=4



LSD_{0.05} for g_s of H_2O = 6.5

LSD_{0.05} for g_s of CO_2 = 10.1

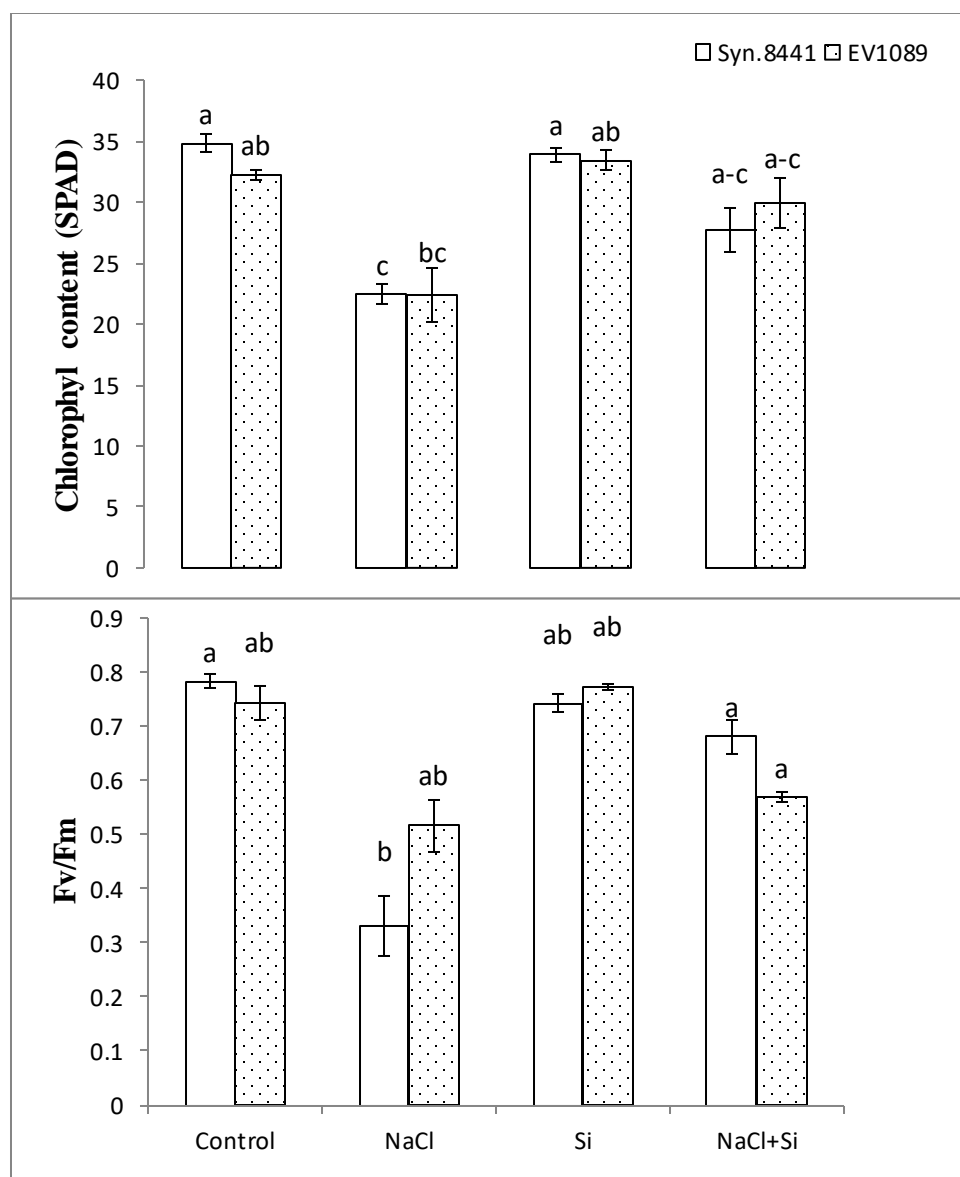
Figure 7.9 Stomatal conductance (g_s) of H_2O and stomatal conductance (g_s) of CO_2 inside maize leaf with and without Si application under salt stress. Bars sharing similar letters are statistically similar to each other at $p \leq 0.05$. Letters belong to each parameter separately and values are means \pm S.E $n=4$



LSD_{0.05} for A/E= 3.6

LSD_{0.05} for A/g_s= 1.21

Figure 7.10 Intrinsic/ Photosynthetic water use efficiency of maize with and without Si application under salt stress. Bars sharing similar letters are statistically similar to each other at $p \leq 0.05$. Letters belong to each parameter separately and values are means \pm S.E n=4



LSD_{0.05} for SPAD= 7.3

LSD_{0.05} for Fv/ Fm= 0.35

Figure 7.11 Chlorophyll content (SPAD) and mean values of maximum quantum yield of primary photochemistry (Fv/Fm) of maize with and without Si application under salt stress. Bars sharing similar letters are statistically similar to each other at $p \leq 0.05$. Letters belong to each parameter separately and values are means \pm S.E n=4

Table 7.1 Root and Shoot Na, K concentrations of maize cultivars influenced by the Si application under salt stress developed after plants established in nutrient solution in pots. Values are means \pm S.En=4 (conc.= concentration; Syn8441= Syngenta8441)

| | Cultivars | Control | NaCl | Si | NaCl+Si |
|---|------------------|----------------|----------------|----------------|-----------------|
| Root Na conc. (mg g⁻¹) | EV1089 | 1.5 \pm 0.2 | 1.9 \pm 0.2 | 1.2 \pm 0.04 | 3.3 \pm 0.2 |
| | Syn.8441 | 2.1 \pm 0.08 | 2.2 \pm 0.3 | 2.3 \pm 0.01 | 3.0 \pm 0.1 |
| Shoot Na conc. (mg g⁻¹) | EV1089 | 0.8 \pm 0.05 | 5.2 \pm 0.3 | 0.8 \pm 0.01 | 4.9 \pm 0.05 |
| | Syn.8441 | 1.5 \pm 0.01 | 1.9 \pm 0.07 | 1.2 \pm 0.05 | 3.3 \pm 0.11 |
| Root K conc. (mg g⁻¹) | EV1089 | 2.1 \pm 0.02 | 0.4 \pm 0.04 | 1.5 \pm 0.09 | 1.2 \pm 0.07 |
| | Syn.8441 | 1.9 \pm 0.06 | 0.4 \pm 0.05 | 2.3 \pm 0.01 | 1.3 \pm 0.04 |
| Shoot K conc. (mg g⁻¹) | EV1089 | 5.6 \pm 0.06 | 2.2 \pm 0.1 | 5.3 \pm 0.15 | 2.9 \pm 0.15 |
| | Syn.8441 | 6.4 \pm 0.06 | 2.6 \pm 0.2 | 6.7 \pm 0.1 | 6.1 \pm 0.003 |

(LSD_{0.05} Root Na 0.8; Shoot Na 1.2; Root K 1.01; Shoot K 2.8)

7.4 Discussion

Salinity stress caused a significant growth reduction in maize crop (Figure 7.1); however interestingly, no significant effects of salinity were observed on dry matter yield of both cultivars when crops were grown in sand culture experiment (Figure 7.10). This might be due to the effect of salinity on germination in pots and ultimately early vegetative growth of cultivar EV 1089; while in sand culture, salinity stress was applied when plants were 18 days old so plants can survive from NaCl stress in their initial growth days. Maize is sensitive to salt stress at early growth stages but tolerant at later stages (Khatoon et al., 2010). There are many possible mechanisms including cytoplasmic toxicity due to Na^+ and/or Cl^- ; insufficient osmotic adjustment, stomatal closure resulting in reduced net photosynthesis; sub-optimal levels of K^+ (or other mineral nutrients) required for maintaining enzyme activities; possible damage from reactive oxygen species; or changes in hormonal concentrations (Flowers et al., 2015). The over production of reactive oxygen species (ROS) in plants is toxic and cause damage to the subcellular organelles like proteins, carbohydrates, lipids and DNA which ultimately leads to the cell death (Tseng et al., 2007). There are different mechanisms adapted by plants to cope up ROS and maintain their healthy growth in saline environment like production of antioxidant enzymes. Salinity stress reduced SOD, CAT and APX production in both maize cultivars; but Si application enhanced their contents under salinity stress (Figure 7.7, 7.8). Superoxide dismutase is important in plant because superoxide radical O_2^\bullet is dismutated by SOD which is reduced to H_2O_2 and O_2 (Prashanth et al., 2008; Wang et al., 2010). This H_2O_2 is converted into H_2O by CAT and APX (Lu et al., 2007). Different scientists also reported increase in SOD, CAT and APX production by Si application under salinity stress in different crops like wheat (Gong et al., 2005; Saqib et al., 2005), barley (Tuna et al., 2008) and maize (Moussa, 2006).

There are different mechanisms adapted by higher plants to reduce oxidative damage resulting from salt stress, through the biosynthesis of a cascade of antioxidants. Among them, phenolic compounds such as phenolic acids, flavonoids and proantho-cyanidins play an important role in scavenging free radicals (Waskiewicz et al., 2013). Salinity stress reduces root and shoot total phenolics (Ashraf et al., 2010) as exhibited in both maize cultivars (Figure 7.6). Silicon application enhanced the total phenolic compounds in root and shoot of both maize cultivars

under stress conditions thereby increasing the tolerance against salinity by scavenging free radicals (Waskiewicz et al., 2013).

To get the verification of our solution culture results; we must get close to the nature. Hence, in sand culture experiment, we found that salt stress can restrict CO₂ assimilation rate (*A*) and stomatal conductance (*g_s*) by partial closure of stomata resulting in reduced CO₂ availability to the plant and restricting the CO₂ fixation mechanism (Flexas and Medrano, 2002). There was a detrimental effect of salinity on metabolic process of protein synthesis (Ohad et al., 1985), which leads to photoinhibition and affect carboxylase activity of RuBisCO (Antolin and Sanchez-Diaz et al., 1993). Hence the decrease in *A* and *g_s* in our experiment (Figure 7.2, 7.3) can be described by modification of RuBisCO enzyme activity. Silicon increases salinity tolerance in crops (Tahir et al., 2011; Liang et al., 2007). Similarly in present study, application of Si increased growth parameters of both cultivars. Different mechanisms have been proposed to explain Si mediated mitigation of salt stress in photosynthetic apparatus of maize crop (Mateos-Naranjo et al., 2013). Silicon application proved to be beneficial in *A* and *g_s* of both maize cultivars; Liang et al. (2005) also described the improvement in *A* and *g_s* due to increased activity of RuBisCO in barley crop under salt stress.

Transpiration rate (*E*) was reduced with Si application under salinity stress (Figure 7.2). This might be due to Si deposition beneath the cell wall of roots (Yeo et al., 1999; Liang et al., 1998) which hinders the translocation of salts and free water movement through xylem. Gong et al. (2005) also reported in rice that higher contents of Si might reduce Na uptake and also cause restriction in bypass flow of water to maintain water status inside plant body. The water use efficiency (WUE) was reduced in both maize cultivars due to applied salinity stress (Figure 7.4); as plant growth faces initial osmotic adjustment (Zhu, 2001). The WUE was improved with Si application under salinity stress in both cultivars; there might be two possible reasons for this increase; either the reduced transpiration is directly related to plant water status (Savant et al., 1999) or the deposition of Si crystals under the epidermal layer of leaves (Raven, 2001) reducing the water loss through stomata or cuticle.

Chlorophyll a and b contents were also decreased with applied salinity stress (Figure 7.5). This might be due to the chlorophyll degradation; it occurs when different proteolytic enzymes such as chlorophyllase formation started due to stress condition (Sabater and

Rodriguez, 1978). The other reason is inefficient activity of photosynthetic apparatus (Mateos-Naranjo et al., 2013). Silicon addition enhances the contents of both pigments under salt stress conditions (Moussa, 2006) as it was confirmed in this study (Figure 7.5).

The photochemical efficiency of photosystem II (Fv/Fm) demonstrated lower values in saline treatment endorsing the results of several previous studies (Al-Agherbay et al., 2004; Ogaya et al., 2011). But very few studies reported about the Si role on (Fv/Fm) under salinity stress. Salt stress reduces the maximum quantum yield of primary photochemistry whose proper working is essential for electron transport chain in chloroplast and mitochondria (Ogaya et al., 2011). There was increase in photochemical efficiency of tomato leaves observed (Al-Agherbay et al., 2004) with Si application under saline conditions; as exhibited in both maize cultivars (Figure 7.13). It might be due to the Si role in detoxifying ROS enhanced under salt stress; as a result chlorophyll increases which lead to the improve (Fv/Fm). According to Maxwell and Johnson (2000), the decrease in value of (Fv/Fm) with salt application and increase in (Fv/Fm) with Si application under abiotic stress might be due to less photoinhibition; as revealed in both maize cultivars (Figure 7.13).

Salinity stress alters the nutritional balance by enhancing Na concentration and decreasing K in both root and shoot of maize cultivars (Table 7.1). Silicon helped plants to uptake nutrients essential for growth under abiotic stress conditions as Si treated plants at high salinity level had higher mineral nutrient concentrations in their tissues such as phosphorus (Mateos-Naranjo et al., 2013). Silicon also worked as a plant Na⁺ detoxification by increasing cell-wall Na⁺ binding in both salt-resistant wheat genotype SARC-1 and salt sensitive 7-Cerros (Saqib et al., 2005). Liang et al. (2005) investigated that Si application increased K conductivity in salt stressed barley cultivars by activating the root plasma membrane H-ATPase pump; similar results were exhibited in shoot K concentration of cultivar EV 1089 (Table 7.1).

Similarly, a negative correlation was found among shoot Na concentration with shoot K concentration and shoot dry matter yield (Figure 7.14). Munns et al. (2006) reported shoot K accumulation decreased in salinity stress which might be due to specific ion toxicity. It is well documented that addition of NaCl to the growing medium decreased the shoot dry matter yield and having a significant negative correlation with K concentration in plants (Hu and Schmidhalter, 2005); as reported in our experiment (Figure 7.14).

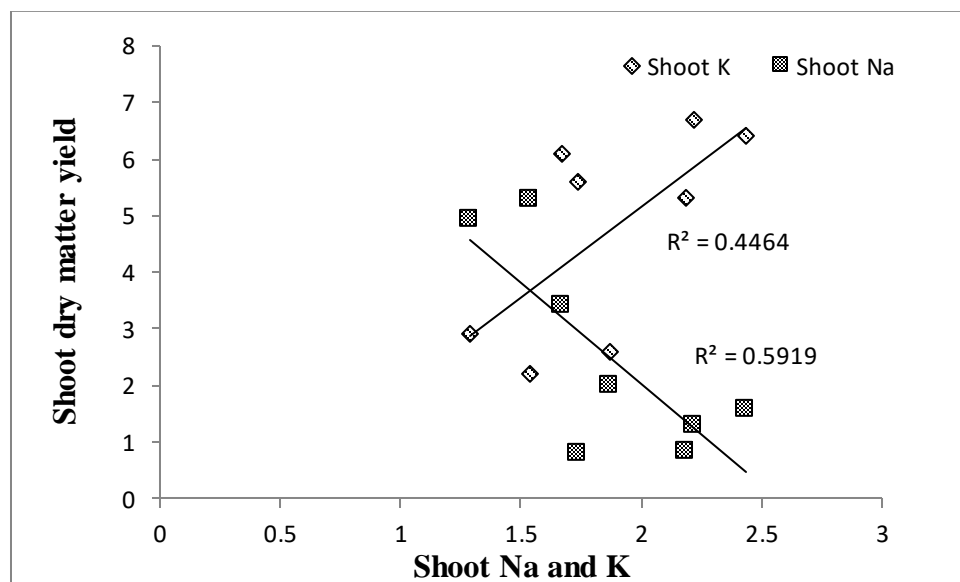


Figure 7.12 Relationship (correlation coefficient, r) of maize shoot Na and K concentrations with shoot dry matter (SDM).

7.5 Conclusion

Salinity stress imposes detrimental effects on both maize cultivars by reducing the activity of antioxidant enzymes and photosynthetic apparatus. It also reduces the dry matter yield and root, shoot K concentrations; ultimately leads to poor growth of plants. Silicon application improved all parameters under salt stress in both maize cultivars by enhancing the K concentration in root and shoots, increasing the plant secondary metabolites (root and shoot total phenolics), and activity of antioxidant enzymes and improving the photosynthetic apparatus. Silicon treated plants have higher photochemical efficiency of photosystem II which leads to healthy growth under salt stress conditions. In a nutshell, Si treated maize plants have better chance to survive under salt stress conditions as their physiological and biochemical apparatus is working far better than non-Si treated plants. Thus, Si application would be beneficial for maize cultivars grown under salt stress conditions and its beneficial effects should be tested on a larger scale i-e field conditions.

Response of salt stressed maize to applied silicon under field conditions

Abstract

The primary objective of the present study was to evaluate the relative suitability of different Si application methods for increasing grain yield and maize plant biomass in salt affected soils. A field study was carried out in which selected salt sensitive and tolerant maize cultivars were sown with different silicon rates at two different locations. The normal ($EC = 2.2 \text{ dS m}^{-1}$) and saline fields ($EC = 7.9 \text{ dS m}^{-1}$) at Okara district was selected for the study. There was significant genotypic variation shown by both cultivars with applied salinity stress. Silicon application either soil or double foliar showed best results in saline and non-saline fields. Salt sensitive cultivar EV 1089 clearly showed poor growth as no biomass yield was obtained in cultivar EV 1089 at control and foliar Si application at twelve leaf stage under saline condition. Maximum grain yield, 100 grain weight and cob weight in saline conditions by double foliar Si application confirmed that foliar application at critical stages also helped salt tolerant cultivar 'Syngenta 8441' to accomplish healthy reproductive stage. This study implies that soil Si application prove to be best for plants grown under saline field condition while foliar application of Si is an economically viable strategy for maize crop; if we remain focus on the application of foliar Si at critical growth stages of maize crop.

8.1 Introduction

Nelson and Mareida, (2001) estimated that about 12 million ha of irrigated land may have gone out of production as a result of salinization. As a result, food insecurity may occur; as population explosion demands more food. According to FAO (2015), about 795 million people are undernourished including majority of the people from central Africa, central Asia and western Asia. Approximately, 15.7% people of south Asia including Pakistan are undernourished (FAO, 2015) due to poverty, no access to better nutrition, urbanization of arable land and degradation of cultivable land i-e salinity, sodicity. This issue can be tackled by the use of salt affected lands so more non cultivated area brought under cultivation leads to more production of food.

Maize is an important cereal crop of Pakistan which grows in most parts of the country due to its large climatic adaptability and short growth duration. Salinity stress significantly affects maize growth and yield and in salt stress, Na^+ and Cl^- are the major toxic ions for maize growth (Moussa, 2006). Plant cells and tissues have to develop the capacity to continue their function in smooth way without major injuries while containing high internal Na and Cl concentrations can be described by the term 'tissue tolerance' (Flowers et al., 2015). Rice genotypes showed severity in chlorosis; as the concentration of Na^+ increased in leaves, there was 50% reduction in leaf chlorophyll lead to low tissue tolerance (Yeo et al., 1987). There are many techniques used by the plant breeders like gene manipulation and its modification, genetic hybridization and gene expression to increased tissue tolerance. Tissue tolerance can be increased by the introduction of mineral nutrient (Si) that can bind Na^+ and enhance K activity.

Silicon is the most prevalent nutrient after oxygen in earth crust (Epstein, 1999) and can make up the plant body as much as 0.1-10%. Its availability in plant body remains satisfactory in normal conditions and no exogenous application of Si is required by plant to complete its life cycle (Epstein and Bloom, 2005). But in stress conditions, one cannot deny the fact that Si is quasi essential for plant. It is involved in the apoplastic binding of different metals like Aluminum in maize (Wang et al., 2004) and manganese in cowpea (Iwasaki et al., 2002a).

Silicon-salinity interaction has been investigated by many scientists in different plant species like barley (Liang et al., 2003), wheat (Saqib et al., 2008; Tahir et al., 2012, 2011), rice (Yeo et al., 1999; Gong et al., 2006; Shi et al., 2013), tomato (Al-Aghabary et al., 2004), canola (Hashemi et al., 2010), cucumber (Zhu et al., 2004), and sugarcane (Ashraf et al., 2010). It decreases the chloride transport to the shoot by minimizing the transpirational bypass flow in rice roots (Shi et al., 2013). Among the yield components, it is involved in enhancing the ripened grains percentage in barley and rice plants under water stress (Ma and Takahashi, 2002). Silicon salt is not a cheap source to apply in field conditions alone as a soil amendment, so different methods like soil and foliar Si applications are used to make it economically viable strategy. Foliar Si application has already been used to combat heavy metal toxicity like cadmium in pots (Liu et al., 2009), but no such study was yet reported on soil and foliar Si application to reduce salt toxicity in maize under field conditions. We screened out salt tolerant and sensitive maize cultivars at germination and vegetative growth stages on the basis of their Si uptake ability in our lab

experiments. Therefore, the primary objective of the present study was to evaluate the relative suitability of different Si application methods for increasing grain yield and maize plant biomass in salt affected soils.

8.2 Materials and methods

A field experiment was carried out at University of Agriculture, Faisalabad farms, Haveli lakha (25° 954' N; 48° 092' E), Punjab, Pakistan. There were two plots selected i-e saline and non-saline. The soil (0-15 cm) was collected from saline and non saline plots of UAF farm research area. The collected soil was sieved through a 2.0 mm sieve. For various physico-chemical properties of soil, a sub sample of the prepared soil was analyzed for pH, EC, extractable Na and K (Richards, 1954) and extractable Si (Elliot and Synder, 1991).

Table 8.1 Physico-chemical properties of soil used for field experiment

| Soil Parameters | Saline Field | Non Saline Field |
|--|--------------|------------------|
| pH | 8.53 | 7.89 |
| ECe (dS m ⁻¹) | 7.9 | 2.02 |
| Extractable Na (mg kg ⁻¹) | 108 | 96 |
| Extractable K (mg kg ⁻¹) | 52 | 71 |
| Extractable Si (mg kg ⁻¹) | 0.48 | 0.61 |

There were two beds plot⁻¹ with net plot size 3×3 m² prepared in both saline and non-saline field and thirty healthy, viable seeds of two selected cultivars (Syngenta 8441 and EV1089) were sown. Silicon was applied in H₂SiO₃ solution form as a soil and foliar treatment at various growth stages of the maize crop. The treatment plan was:

1. Control
2. Si₁= 1 mM (250 g plot⁻¹) Soil Si application
3. Si₂= 1 % (0.36 L plot⁻¹) Foliar Si application 30 days after maize was sown (six leaf stage);

4. $Si_3 = 1 + 1\%$ (0.72 L plot^{-1}) Double Foliar Si application 30 and 50 days after maize were sown (six and twelve leaf stages);
5. $Si_4 = 1\%$ (0.36 L plot^{-1}) Foliar Si application 50 days after maize was sown (twelve leaf stage).

Each treatment was replicated four times and randomized accordingly. Irrigation water was maintained in both fields at field capacity. Basic doses of N, P and K were applied at the rate of 200, 150 and 100 kg ha⁻¹ as urea, DAP and SOP, respectively. The crop was harvested at maturity and different growth and yield related traits were recorded.

Statistical analysis

Data of both studies was statistically analyzed by *Microsoft Excel 2010*[®] (Microsoft Cooperation, USA) and *Statistix 8.1*[®] (Analytical Software, Tallahassee, USA). Significantly different treatment means were separated using least significant difference (LSD) test (Steel et al., 1997).

8.3 Results

8.3.1 Straw yield

Growth performance of cultivar EV1089 was better than Syngenta8441 at non-saline conditions, however this cultivar could not withstand salinity stress and almost all plants wilted when grown in saline soil (Figure 8.1). Hence, No straw yield (SY) was obtained in some plots of cultivar EV1089 under saline treatment. Silicon application significantly ($p > 0.05$) improved straw yield in both maize cultivars irrespective of application methods however maximum increase in SY relative to saline treatment was observed when Si was applied as soil application. There was two-fold increase in straw yield of cultivar ‘Syngenta8441’ observed with soil Si application under salinity stress compared to control.

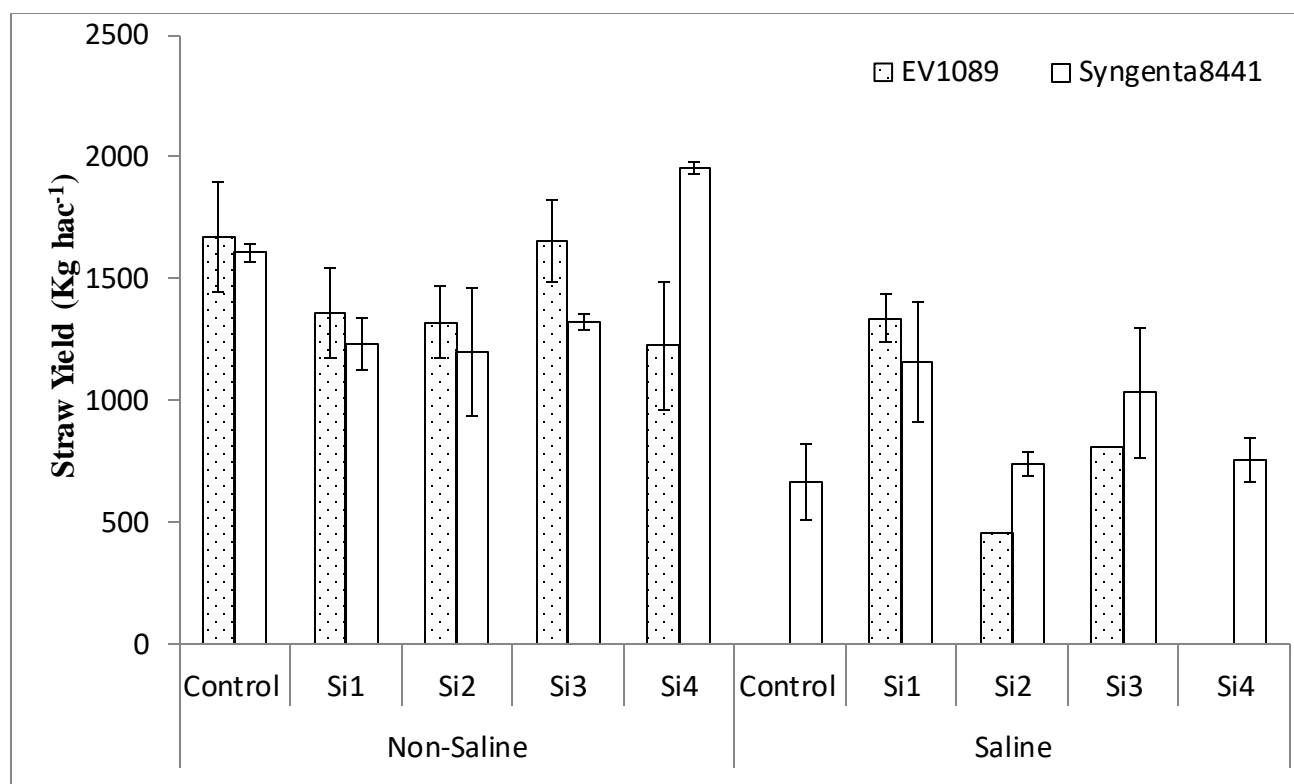
8.3.2 Grain yield

Among yield components, grain yield (GY) of cultivar EV1089 was better than Syngenta 8441 at non-saline conditions, however this cultivar could not withstand salinity stress and the cobs eventually lost their grain size and shape when grown in saline soil (Figure 8.2). Salinity stress significantly ($p > 0.05$) reduced GY in both cultivars; however reduction was

variable among cultivars. Maximum reduction was observed in cultivar EV 1089 where no grain yield obtained in some plots under salinity stress, while cultivar Syngenta8441 showed minimum reduction in GY. Silicon addition significantly decreased the reduction in GY under salinity stress compared to saline treatment only. Cultivar ‘Syngenta 8441’ showed two fold increase in grain yield with double foliar Si application under salinity stress relative to its control.

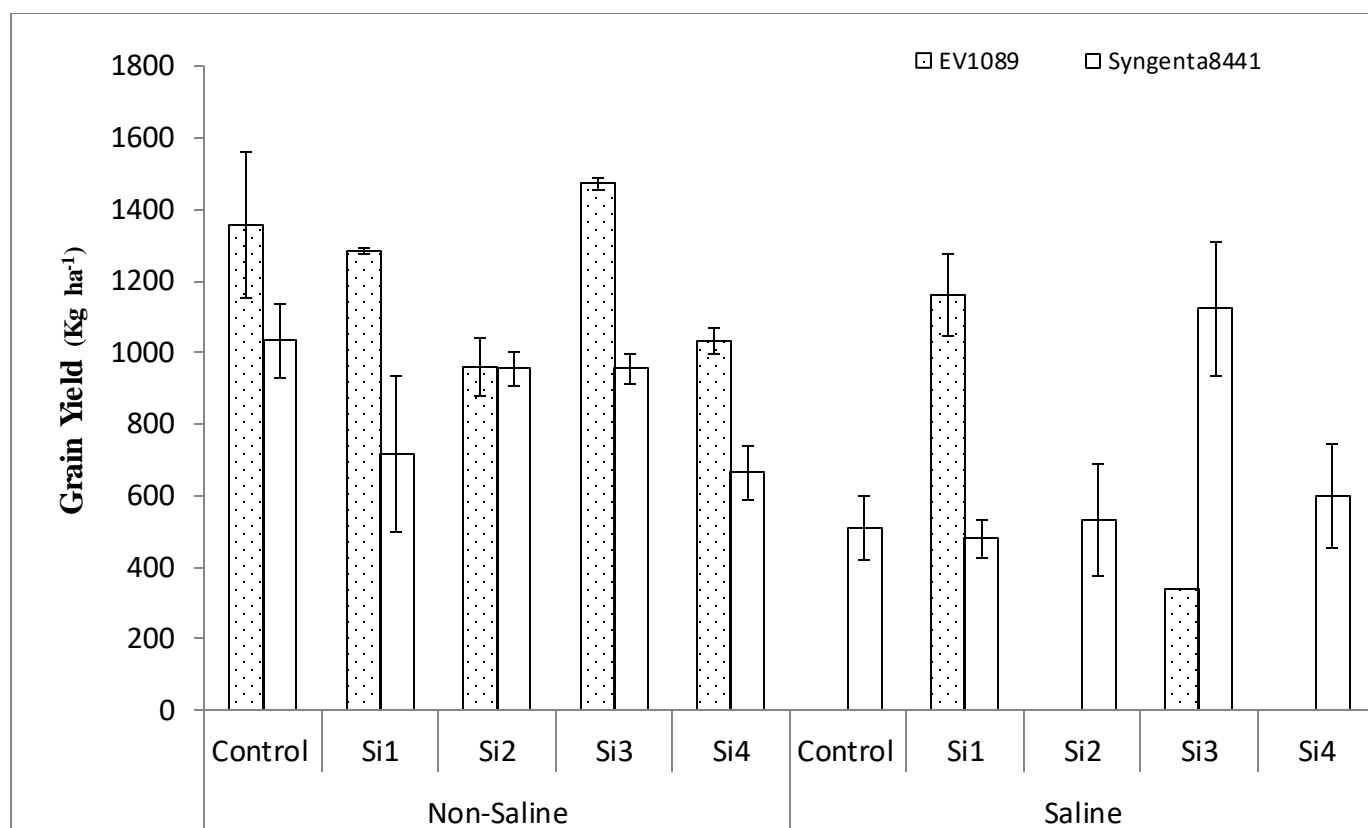
8.3.3 Number of grains cob⁻¹

Similarly, Number of grains cob⁻¹ of Cultivar EV1089 was better than Syngenta8441 at non-saline conditions, however this cultivar could not withstand salinity stress and many cobs eventually lost their grains when grown in saline soil (Figure 8.3). Salinity stress significantly ($p>0.05$) reduced number of grains cob⁻¹ in both cultivars; however reduction was variable among cultivars. Silicon application significantly enhanced the number of grains cob⁻¹ under saline conditions; where 43 and 26% increase in number of grains cob⁻¹ observed in cultivar ‘Syngenta-8441’ with double foliar Si application and Si application at twelve leaf stage under saline conditions relative to control. Maximum increase in number of grains cob⁻¹ (6 folds) was observed in cultivar EV1089 with soil Si application in saline field relative to control.



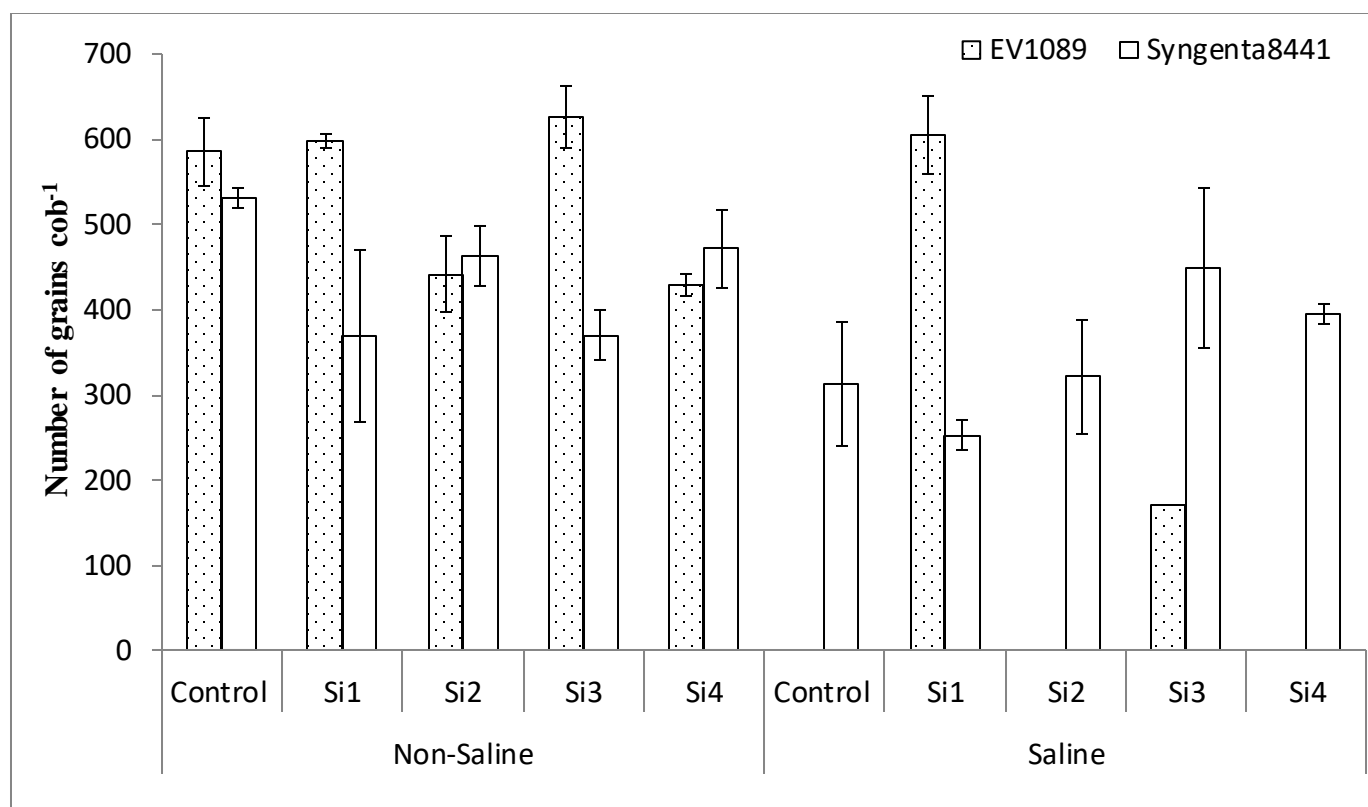
(LSD_{0.05}: interaction effect for Non-Saline, 752; for Saline, 532)

Figure 8.1 Straw yields of maize cultivars influenced by the Si application under salt stress in field conditions. Values are means \pm S.E. $n=4$. (Si1= Soil Si application; Si2= Foliar Si application 30 days after maize were sown; Si3= Double Foliar Si application 30 and 50 days after maize were sown; Si4= Foliar Si application 50 days after maize were sown).



(LSD_{0.05}: interaction effect for Non-Saline, 486; for Saline, 682)

Figure 8.2 Grain yields of maize cultivars influenced by the Si application under salt stress in field conditions. Values are means \pm S.E. n=4. (Si1= Soil Si application; Si2= Foliar Si application 30 days after maize were sown; Si3= Double Foliar Si application 30 and 50 days after maize were sown; Si4= Foliar Si application 50 days after maize were sown).



(LSD_{0.05}: interaction effect for Non-Saline, 197; for Saline, 221)

Figure 8.3 Number of grains cob⁻¹ of maize cultivars influenced by the Si application under salt stress in field conditions. Values are means \pm S.E. n=4. (Si1= Soil Si application; Si2= Foliar Si application 30 days after maize were sown; Si3= Double Foliar Si application 30 and 50 days after maize were sown; Si4= Foliar Si application 50 days after maize were sown).

8.3.4 Biological yield

Maize biological yield was significantly ($p>0.05$) influenced by the main effects of cultivars and Si application rates in saline conditions (Figure 8.4). Growth performance of cultivar EV 1089 was better than Syngenta 8441 at non-saline conditions, however this cultivar could not withstand salinity stress and almost all plants wilted when grown in saline soil. Hence, No biological yield (BY) was obtained in some plots of cultivar EV1089 under saline treatment. Silicon application significantly ($p>0.05$) improved BY in both maize cultivars irrespective of application methods however maximum increase in BY relative to saline treatment was observed when Si was applied as soil application. Cultivar Syngenta 8441 showed 3-fold increase in maize BY with soil Si application (Si_1) and double foliar Si application (Si_3) compared to control under saline conditions.

8.3.5 Harvest index

Harvest index was significantly ($P\leq 0.05$) influenced by the main effects of cultivars and the interactive effects of cultivars and Si application (Figure 8.5). Growth performance of cultivar EV1089 was better than Syngenta8441 at non-saline conditions, however this cultivar could not withstand salinity stress and almost all plants wilted when grown in saline soil. Hence, No harvest index (HI) was obtained in some plots of cultivar EV1089 under saline treatment. Silicon application significantly ($p>0.05$) improved HI in cultivar EV1089 with Soil and double foliar Si application methods however maximum increase in HI relative to saline treatment was observed when Si was applied as soil application in cultivar EV1089. The reduction in HI of cultivar EV1089 decreased from 0 to 3 folds when soil Si was applied under saline conditions relative to its control.

8.3.6 100 grain weight

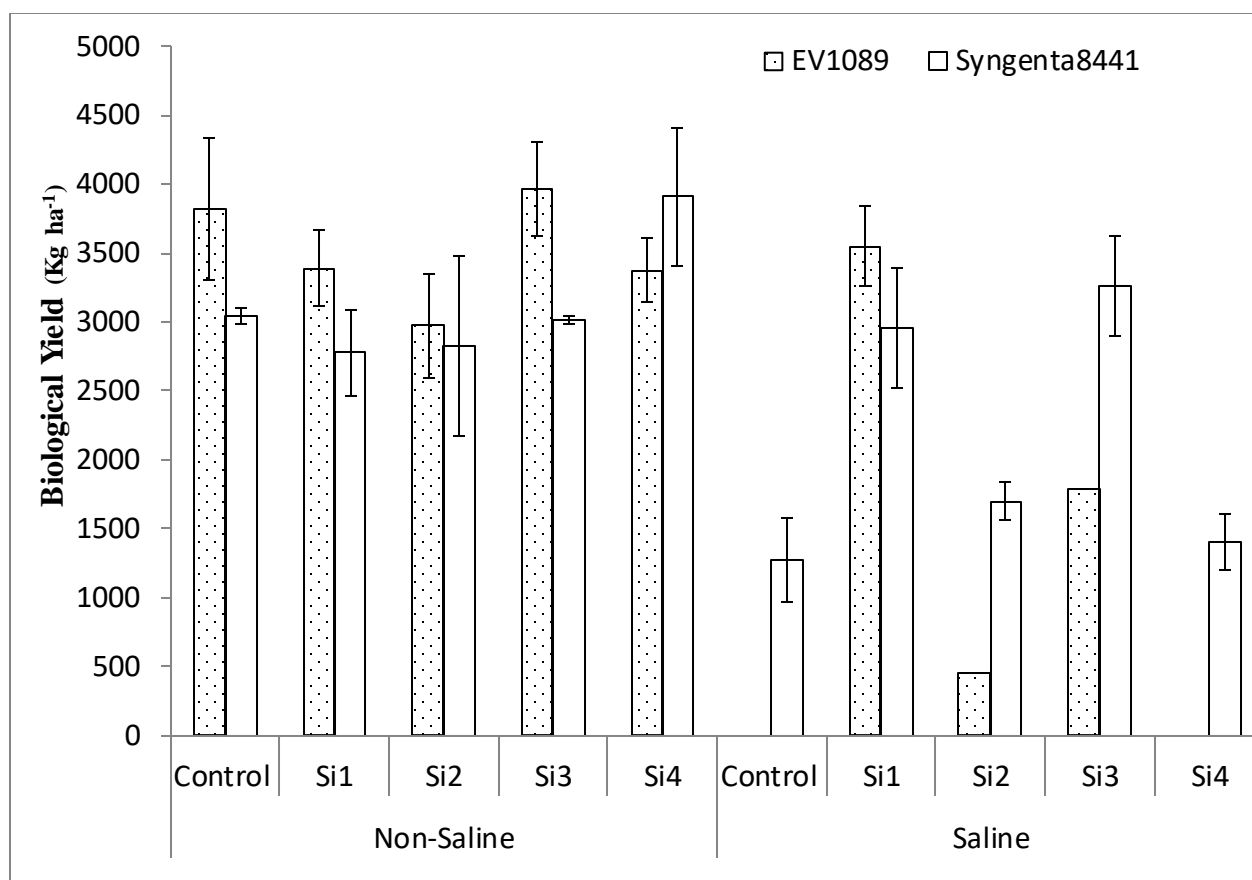
The 100 grain weight in maize cultivars ranged from 21.2 to 29.2 g plant⁻¹ in non-saline and 16.8 to 23.8 g plant⁻¹ in saline field at various Si application rates (Table 8.2). Growth performance of cultivar EV 1089 was better than Syngenta 8441 at non-saline conditions, however this cultivar could not withstand salinity stress and many cobs lost their grain weight when grown in saline soil. Hence, No 100 grain weight was obtained in some plots of cultivar EV1089 under saline treatment. Silicon application significantly ($p>0.05$) improved 100

grain weight in both maize cultivars irrespective of application methods however maximum increase in BY relative to saline treatment observed when Si was applied as double foliar application.

There were 27 and 13% increase in 100 grain weight observed in Syngenta 8441 with double foliar Si application and soil Si application relative to control in saline field.

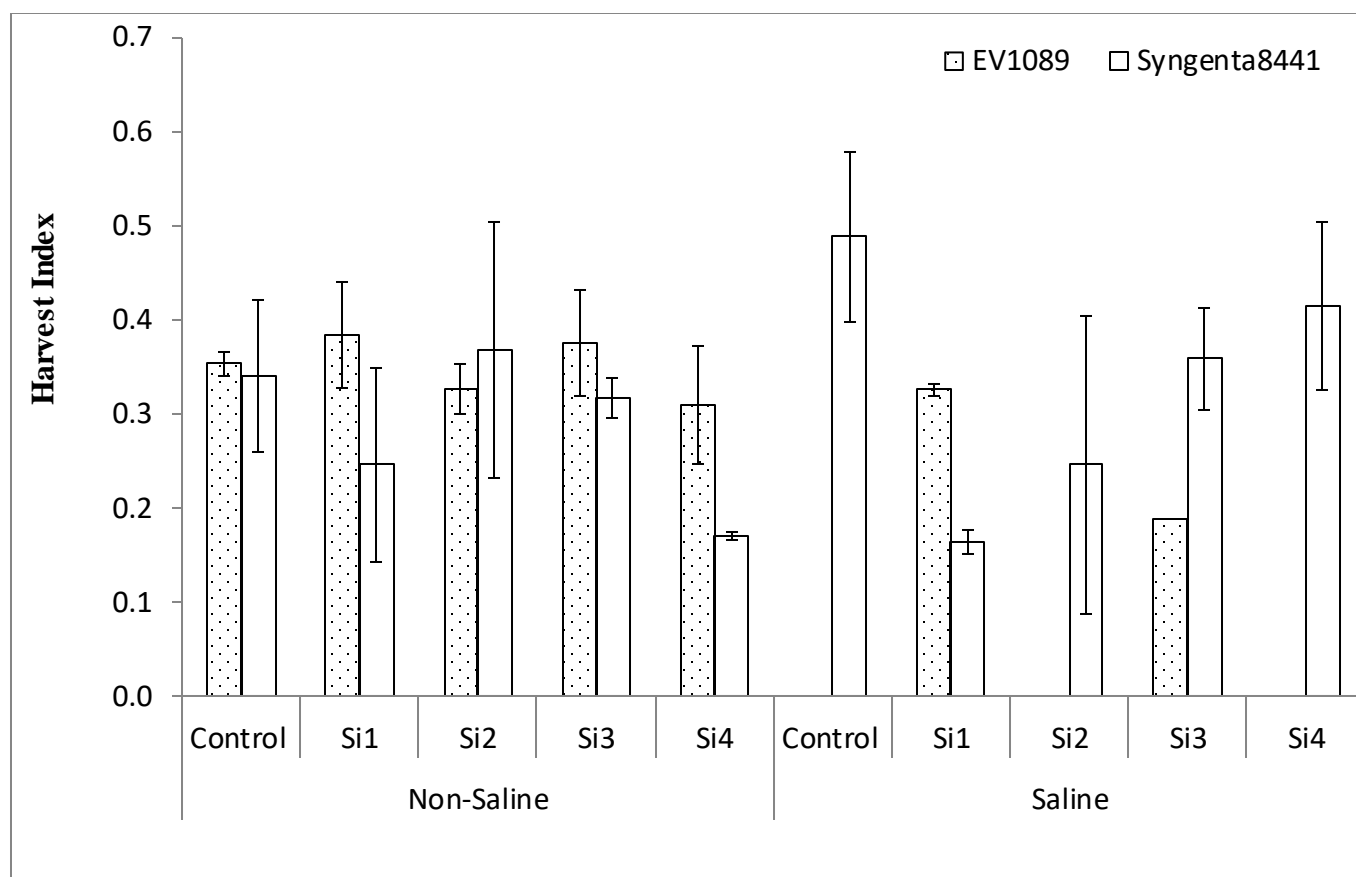
8.3.7 Cob weight

Cob weight was significantly ($P \leq 0.05$) influenced by the main effects of cultivars and Si application in saline field (Table 8.2). Growth performance of cultivar EV 1089 was better than Syngenta 8441 at non-saline conditions, however this cultivar could not withstand salinity stress and many cobs lost their weight when grown in saline soil. Hence, No cob weight was obtained in some plots of cultivar EV1089 under saline treatment. Silicon application significantly ($p > 0.05$) improved cob weight in both maize cultivars irrespective of application methods however maximum increase in cob weight relative to saline treatment observed when Si was applied as soil application. Cultivar ‘Syngenta 8441’ shown 2 fold increase in cob weight with Soil and double foliar Si application relative to control in saline field.



(LSD_{0.05}: interaction effect for Non-Saline, 1681; for Saline, 1682)

Figure 8.4 Biological yields of maize cultivars influenced by the Si application under salt stress in field conditions. Values are means \pm S.E. n=4. (Si1= Soil Si application; Si2= Foliar Si application 30 days after maize were sown; Si3= Double Foliar Si application 30 and 50 days after maize were sown; Si4= Foliar Si application 50 days after maize were sown).



(LSD_{0.05}: interaction effect for Non-Saline, 0.15; for saline, 0.33)

Figure 8.5 Harvest Index of maize cultivars influenced by the Si application under salt stress in field conditions. Values are means \pm S.E. n=4. (Si1= Soil Si application; Si2= Foliar Si application 30 days after maize were sown; Si3= Double Foliar Si application 30 and 50 days after maize were sown; Si4= Foliar Si application 50 days after maize were sown).

8.3.8 Number of lines cob⁻¹

Number of lines cob⁻¹ in maize cultivars ranged from 12 to 16 rows in non-saline and saline fields (Table 8.3). There was no formation of cob occur in cultivar EV 1089 when grown in saline soil as this cultivar could not withstand salinity stress. Hence, No number of lines cob⁻¹ was obtained in some plots of cultivar EV1089 under saline treatment. Silicon application significantly ($p>0.05$) improved number of lines cob⁻¹ in both maize cultivars irrespective of application methods however maximum increase in cob weight relative to saline treatment observed when Si was applied as soil application.

8.3.9 Plant height

Plant height was significantly ($P\leq0.05$) influenced by the main effects of cultivars and Si application in saline field (Table 8.3). No plant height was obtained in some plots of cultivar EV1089 under saline treatment. Silicon application significantly ($p>0.05$) improved plant height in both maize cultivars irrespective of application methods however maximum increase in plant height relative to saline treatment observed when Si was applied as soil application. Cultivar Syngenta8441 showed 35% increase in plant height when Si was applied as soil applied in saline field relative to saline treatment only.

Table 8.2 100 grain weight (g plant⁻¹) and cob weight (kg ha⁻¹) of maize cultivars influenced by the Si application under salt stress in field conditions. Values are means \pm S.E. n=4. (Si1= Soil Si application; Si2= Foliar Si application 40 days after maize were sown; Si3= Double Foliar Si application 40 and 60 days after maize were sown; Si4= Foliar Si application 60 days after maize were sown)..

| | 100 Grain Weight (g) | | | Cob Weight (Kg ha ⁻¹) | |
|-------------------|-------------------------|----------------|-----------------|--------------------------------------|----------------|
| | | | | | |
| | Treatments | EV-1089 | Syng 8441 | EV-1089 | Syn-8441 |
| Non-Saline | Control | 25.7 \pm 4.3 | 21.8 \pm 3.4 | 2144 \pm 581 | 1437 \pm 183 |
| | Si1 | 24.1 \pm 1 | 21.2 \pm 1.6 | 2028 \pm 180 | 1544 \pm 408 |
| | Si2 | 24.5 \pm 0.7 | 23.3 \pm 1.2 | 1650 \pm 447 | 1627 \pm 388 |
| | Si3 | 26.6 \pm 3.5 | 29.2 \pm 1.9 | 2311 \pm 345 | 1688 \pm 0 |
| | Si4 | 27.1 \pm 0.1 | 16.5 \pm 6.7 | 2144 \pm 62 | 1955 \pm 958 |
| | | | | | |
| Saline | Control | 0 | 18.7 \pm 2.2 | 0 | 605 \pm 290 |
| | Si1 | 21.4 \pm 1.1 | 21.2 \pm 1.6 | 2211 \pm 377 | 1655 \pm 565 |
| | Si2 | 0 | 16.69 \pm 9.4 | 0 | 955 \pm 377 |
| | Si3 | 22.2 | 23.8 \pm 7.3 | 978 | 1672 \pm 691 |
| | Si4 | 0 | 16.8 \pm 7.4 | 0 | 638 \pm 227 |
| | | | | | |

For 100 grain weight (LSD_{0.05}: interaction effect for non-saline, 7.0; for saline, 15)

For cob weight (LSD_{0.05}: interaction effect for non-saline, 1095; for saline, 1041)

Table 8.3 Number of lines cob^{-1} and plant height (cm) of maize cultivars influenced by the 1mM Si application under salt stress in field conditions. Values are means \pm S.E. $n=4$. (Si1= Soil Si application; Si2= Foliar Si application 30 days after maize were sown; Si3= Double Foliar Si application 30 and 50 days after maize were sown; Si4= Foliar Si application 50 days after maize were sown). EC of saline Field= 7.9 dSm^{-1}

| | | Number of lines (cob^{-1}) | | Plant Height (cm) | |
|------------|---------|--|--------------|----------------------|----------------|
| Treatments | | EV1089 | Syn8441 | EV1089 | Syn8441 |
| Non-Saline | Control | 14 \pm 0 | 14 \pm 0 | 158 \pm 25 | 144 \pm 13.9 |
| | Si1 | 16 \pm 1 | 15 \pm 0.3 | 149 \pm 0 | 155 \pm 15 |
| | Si2 | 14 \pm .3 | 16 \pm 0.3 | 172 \pm 1 | 166 \pm 7.5 |
| | Si3 | 13 \pm 0.7 | 12 \pm 0 | 150 \pm 7 | 134 \pm 19.3 |
| | Si4 | 16 \pm 0.3 | 14 \pm 1 | 148 \pm 18 | 153 \pm 1 |
| Saline | Control | 0 | 13 \pm 0.7 | 0 | 126 \pm 7.5 |
| | Si1 | 16 \pm 0.5 | 13 \pm 0.7 | 152 \pm 0 | 170 \pm 4.3 |
| | Si2 | 0 | 16 \pm 0.3 | 106 \pm 0 | 122 \pm 19.2 |
| | Si3 | 12 \pm 1 | 14 \pm 0.7 | 140 \pm 0 | 147 \pm 3.2 |
| | Si4 | 0 | 16 \pm 0 | 0 | 137 \pm 4.3 |

For no. of lines cob^{-1} (LSD_{0.05}: interaction effect for non-saline, 2.8; for saline, 6.4)

For Plant height (LSD_{0.05}: interaction effect for non-saline, 62; for saline, 92)

8.4 Discussion

Being glycophyte, maize growth and yield retards severely as salinity increases in soil solution (Khan et al., 2006). About 50% yield reduction has been reported at EC 3.9 dS m⁻¹ (Ayres and Westcot, 1985). Silicon plays its role in minimizing the harmful effects of salinity stress. Silicon application increased the maize crop yield and biomass in both cultivars when applied in salt stressed field (Figure 8.3).

Foliar application of Si significantly increased the dry matter yield of the straw and grains of maize cultivars grown in the salt affected soils; when Si was applied at different growth stages of maize crop (Figure 8.1, 8.2). Salt sensitive cultivar 'EV 1089' started germination in saline field but could not cope up salt stress as time went on due to many factors i-e high atmospheric temperature, absence of Si in control and plots selected for foliar Si application and high osmotic stress. Double foliar Si application (Si₃) presented higher grain yield in salt tolerant maize crop 'Syngenta 8441' relative to other foliar treatments (Figure 8.2) as it not only gives strength to the plant but also help it to initiate reproductive stage. Rahimi et al. (2012) investigated that dry and fresh plant weight, 1000 grain weight and grain yield had no significant influence by NaCl addition when Si was also applied. Similarly, Si₁ at the time of crop sowing provides the best results in both salt stressed maize crops as compared to other treatments. The reason behind that 1 mM Si was present in soil when the germination started; as maize crop is a Si accumulator (Liang et al., 2007) so Si helped it to overcome the stress condition. Silicon made salt dilution by improving the water storage within plant tissues, which allows a higher growth rate that, in turn, mitigating salt toxicity effects (Romero-Aranda et al., 2006). Savvas et al. (2009) also reported that the salinity-associated suppression was alleviated by the inclusion of 1 mM of Si in the salinized solution.

In a comparison of soil vs. foliar Si treatments in salt stressed field; Si₁ gives significant higher grain yield in salt sensitive cultivar 'EV-1089' due to presence of Si in soil thorough out the growing period; as cultivar EV1089 is a salt sensitive cultivar so it requires exogenous mineral nutrition (Si) to cope up salt stress at early growth stages. Similarly, Si₃ gives maximum grain yield in salt tolerant cultivar 'Syngenta 8441'; as cultivar Syngenta 8441 showed proper growth at early growth stages under saline conditions so double foliar application of Si at critical vegetative growth stages helped this cultivar to attain higher

grain yield (Figure 8.2). The different expression of both cultivars with Si application under salt stress can be associated to their genetic variability.

Tuna et al. (2008) reported that Si significantly improved wheat dry biomass when added to the salt treatment especially at the higher salt levels (100 mM NaCl) where reduction in total plant dry weight in the NaCl treatment were 39 and 54% for salt-tolerant (Izmir-85) and sensitive (Gediz-75) cultivars, respectively. Silicon addition mitigated the negative effect of Na⁺ on different growing parts of the crop and enhanced its biomass yield (Al-Aghabary et al., 2004). Similarly, there was no difference in biological yield (Figure 8.3) obtained in cultivar 'EV1089' at control and Si application at twelve leaf stage under salt stress as Si application at twelve leaf stage could not help plants to enter reproductive stage.

8.5 Conclusion

There was significant genotypic variation shown by both cultivars with applied salinity stress. Salt sensitive cultivar 'EV-1089' clearly showed poor growth while salt tolerant cultivar 'Syngenta 8441' showed proper growth under salinity stress. Silicon application either soil or double foliar presented best results in saline and non-saline fields. Cultivar EV1089 showed excellent growth in normal conditions so it must be preferred in normal soils; while cultivar Syngenta 8441 should be grown in salt affected conditions. This study implies that soil Si application proved to be best for plants grown under saline field condition while foliar application of Si is an economically viable strategy for maize crop.

General Discussion

Salinity stress hampers the growth of crop species in general and cereals in particular through osmotic and oxidative stress (Munns et al. 2006; 2008). Maize is known as Si accumulator (Liang et al., 2007); under salt stress conditions, Si helps the maize crop to continue its growth and development (Moussa, 2006). However, very few studies have yet been reported about genetic variations among maize cultivars regarding Si acquisition under salt stress conditions. In the present study, fifteen latest maize cultivars were grown in petriplates against NaCl stress. Cultivars exhibited significant differences in terms of growth performance and the absolute values of different germination parameters of salt sensitive cultivars such as EV1089 and 32B33, were more or less similar or even higher than salt tolerant cultivars, but their performance at saline treatments was very poor.

Exogenous application of mineral nutrition is a healthy strategy to cope up salt stress. Therefore, eight salt sensitive and salt tolerant cultivars were grown in petri plates with Si application under NaCl stress. The response of maize cultivars varied significantly in terms of different germination parameters. The absolute values of seedling length, vitality index, seed vigor of salt sensitive cultivars were more or less similar or even higher, but their performance at saline treatments was very poor. Silicon application increased all germination parameters under salt stress conditions.

Those eight salt sensitive and salt tolerant cultivars were also grown in pots to evaluate different ionic concentrations and to verify our germination trials. Silicon inclusion to a salt stressed maize plant increased the shoot growth at early vegetative growth. It is also reported that Si application enhances root fresh and dry weights in maize under saline regimes (Moussa, 2006; Parveen and Ashraf, 2010).

Salt stress increased the shoot Na concentration while shoot K concentration was decreased (Table 7.1) as already reported in a number of plants (Moussa, 2006; Kafi and Rahimi, 2011; Nasim et al., 2008; Tahir et al., 2012).

The genetic difference can be utilized for the production of more salt tolerant cultivars. The genetic variation among cultivars for salinity tolerance and their variable response to Si application demands further investigation for identification of Si induced mechanism of salinity tolerance in maize. Syngenta-8441 was categorized as salt tolerant and EV-1089 as salt sensitive cultivar. Thus, a hydroponic trial was conducted to study the possible mechanisms mediated by Si under salinity stress. There are different mechanisms adapted by higher plants to reduce oxidative damage resulting from salt stress, through the biosynthesis of a cascade of antioxidants. Among them, phenolic compounds such as phenolic acids, flavonoids and proantho-cyanidins play an important role in scavenging free radicals (Waskiewicz et al., 2013). Salinity stress reduces root and shoot total phenolics (Ashraf et al., 2010) as exhibited in both maize cultivars (Figure 7.7). Silicon application enhanced the total phenolic compounds in root and shoot of both maize cultivars under stress conditions thereby increasing the tolerance against salinity by scavenging free radicals (Waskiewicz et al., 2013).

Salinity stress reduces SOD, CAT and APX production in both maize cultivars (Figure 7.8); but Si application enhance their contents under salinity stress. Superoxide dismutase is important in plant because superoxide radical O_2^{\bullet} is dismutated by SOD which is reduced to H_2O_2 and O_2 (Prashanth et al., 2008; Wang et al., 2010). This H_2O_2 is converted into H_2O by CAT and APX (Lu et al., 2007). Different scientists also reported increase in SOD, CAT and APX production by Si application under salinity stress in different crops like wheat (Gong et al., 2005; Saqib et al., 2005), barley (Tuna et al., 2008) and maize (Moussa, 2006). As reactive oxygen species production and lipid peroxidation are the major consequences of salt stress which further deteriorates the seed (Lehner et al., 2008), so exogenous application of Si can protect the seed against reactive oxygen species and lipid peroxidation, thus enhancing seed germination.

Salt stress also restrict CO_2 assimilation rate (A) and stomatal conductance (g_s) by partial closure of stomata resulting in reduced CO_2 availability to the plant (Figure 7.2) and restricting the CO_2 fixation mechanism (Flexas and Medrano, 2002). Photochemical efficiency of tomato leaves increases (Al-Agherbay et al., 2004) with Si application under saline conditions (Figure 7.13); as exhibited in both maize cultivars. It might be due to the Si role in detoxifying ROS enhanced under salt stress; as a result chlorophyll increases which lead to the improve (F_v/F_m).

Salinity stress alters the nutritional balance by enhancing Na concentration and decreasing K in both root and shoot of maize cultivars. It is well documented that addition of NaCl to the growing medium decreased the shoot dry matter yield and having a significant negative correlation with K concentration in plants (Hu and Schmidhalter, 2005); observed in our experiment.

To get verification of our results, a field trial is conducted. Salt sensitive cultivar 'EV-1089' and salt tolerant cultivar 'Syngenta-8441' were grown in saline and non saline fields. Salt sensitive cultivar 'EV-1089' clearly shown poor growth in saline field where no biomass yield was obtained under control and Si₄ treatment. Soil and foliar Si application increases the growth and yield of both salt stressed maize cultivars. Foliar Si application is a shotgun approach already used by Korndorfer (2004) in normal field conditions and by Liu et al. (2009) in rice plants under Cd toxicity, where Si not only acts as a barrier inside plant body against unfavourable environment but also enhances the biomass yield; as exhibited in our experiment. Soil Si application presented higher grain yield in salt sensitive cultivar 'EV-1089' while double foliar Si application provided higher grain yield in salt tolerant cultivar 'Syngenta-8441'. The different expression of both cultivars with Si application under salt stress can be associated to their genetic variability.

Some of the conclusions drawn from the ongoing discussions are listed below:

1. Significant genetic variation existed among maize cultivars which should be exploited to use salt affected soils.
2. Syngenta-8441 was categorized as salt tolerant and EV-1089 as salt sensitive cultivar.
3. Proper selection of cultivars for normal as well as saline soils would enhance maize yield.
4. Silicon application enhanced germination, growth and improved ionic parameters.
5. Silicon treated plants have higher antioxidants, photosynthetic parameters and photochemical efficiency of photosystem II which leads to healthy growth under salt stress conditions.
6. Cultivar EV1089 showed excellent growth in normal conditions so it must be preferred in normal soils; while Cultivar Syngenta 8441 should be grown in salt affected conditions.
7. Foliar Si application is a viable strategy as salt tolerant cultivar Syngenta 8441 gives maximum biological and grain yield with Si₃ treatment relative to Si₁.

Summary

Food security is a serious issue in developing countries because of ever-increasing population. Salt affected soils are one of the main reasons in increasing food security problems. Rengasamy, (2006) reported that out of 13 billion hectares of total land, one billion is salt affected, including 30% of all irrigated land. Hence, for sustaining food security, a high priority should be given to safe use of salt affected soils. It not only causes ion toxicity and physiological drought, but also reduces water use efficiency and photosynthesis due to interveinal chlorosis which ultimately decreases crop yields. As crop yield and its sustainability is a pre-requisite to flourish the country's economy, it is highly recommended to adopt strategies aiming at increased crop production on salt affected lands.

Judicious use of mineral nutrition is a recommended shotgun strategy as it strengthens the cereals to cope against salt stress. Silicon availability in the soil has never been questionable; as wide range of plants can uptake Si from soil solution freely. It is beneficial nutrient because under normal conditions, plants can complete their growth and development without Si (Epstein, 2009). Increasing the availability of Si in the growth medium can reduce salinity stress in plants by altering soil and plant factors (Kafi and Rahimi, 2011), but specific mechanisms are still debatable. Liang et al. (2007) reported that silicon uptake in a salt stressed plant increases root activity for nutrient uptake, inhibits transpiration which reduces osmotic stress. It also increases the activity of ATPase & PPase in plasma membrane which ultimately increases K and decreases Na uptake (Tuna et al., 2008). The other typical beneficial effects of Si are usually expressed more clearly when plants are subjected to various abiotic and biotic stresses (Ma, 2004). Silicon is probably the only element which is able to enhance the resistance to multiple stresses. In Pakistan, Si based saline soil management is still under process and have not been estimated in the context of Pakistan agriculture.

Therefore, to evaluate the variation in salinity exists among maize growing areas of Punjab and a nutrient indexing of Si in maize crop and associated soils is described in chapter 3. There were 31 soil and plant samples taken at District Sargodha while 19 soil and plant samples taken at District Okara. The results suggested that District Okara soil and plant samples presented higher

concentration of Si relative to District Sargodha samples. Soil pH was higher in both maize growing areas i.e District Sargodha and Okara. District Okara samples have higher EC relative to district Sargodha which leads to the lower K Concentration in maize plant samples. District Okara plant samples have higher Na/K ratio relative to District Sargodha. Hence, District Okara soil and plant samples presented more interesting facts to plan future studies.

In chapter 4 and 5, two independent germination studies were conducted to categorize the latest maize cultivars according to their tolerance against salinity stress and to screen out the salt tolerant and sensitive maize cultivars at germination on the basis of their Si uptake ability. Initially 15 maize cultivars were categorized as sensitive, medium and tolerant to salinity on the basis of germination parameters under control and 90 mM NaCl salinity. Four cultivars were categorized as salt sensitive, while four cultivars were categorized as salt tolerant. These eight cultivars were selected and seeds were germinated in petri plates with 90 mM NaCl and 2 mM K_2SiO_3 . Salinity stress significantly decreased all of germination parameters while Si application increased them however effect was variable among tolerant and sensitive cultivars.

In chapter 6, a pot study was conducted to test different cultivars in soil conditions to evaluate different ionic parameters and to verify the germination trials. There were eight cultivars selected i.e Four salt sensitive maize cultivars (Monsento-919, Golden cross, 32B33 and EV-1089) and four salt tolerant (Syngenta-8441, Pioneer-30R50, ICI hybrid and Dekalb). There were two Si levels (0 and 2 mM) and two levels of salinity (0 and 60 mM NaCl). Each treatment was replicated three times. Shoot K and Si concentration was significantly reduced in salt sensitive cultivar (EV 1089) as compared to salt tolerant (Syngenta 8441) under salt stress. Silicon treated maize plants perform far better than non-treated plants in saline conditions. It alleviated the toxic effect of Na and increased the K concentration in maize shoot. Growth performance of cultivar Syngenta 8441 was least affected by salt stress so it is regarded as salt tolerant cultivar; while cultivar EV 1089 was regarded as salt sensitive.

In chapter 7, two independent experiments were carried out in Pakistan and Austria including two cultivars (Syngenta 8441 and EV 1089) in hydroponic and sand culture solutions, respectively. Salinity stress significantly reduces the production of antioxidant enzymes and total phenolics. Similarly, inefficient working of photosynthetic apparatus including photochemical efficiency of photosystem II and negative correlation of shoot sodium concentration with shoot

potassium and dry matter leads to osmotic stress on maize cultivars. Silicon addition alleviated both osmotic and oxidative stress on maize crop by improving the contents of defensive machinery and water use efficiency. It increased root and shoot potassium concentration and dry weight of whole maize plants. It enhanced maximum quantum yield of primary photochemistry which leads to smooth electron transport chain; ultimately lowers production of reactive oxygen species in chloroplast or mitochondria under salt stress. Therefore, this study implies that silicon treated maize plants have better chance to survive under salt stress conditions as their physiological and biochemical apparatus is working far better than non-silicon treated plants.

In chapter 8, a field study was carried out in which selected salt sensitive and tolerant maize cultivars were sown with different silicon rates at two different locations. The normal ($EC = 2.2 \text{ dS m}^{-1}$) and saline fields ($EC = 7.9 \text{ dS m}^{-1}$) at Okara district was selected for the study. There was significant genotypic variation shown by both cultivars with applied salinity stress. Silicon application either soil or double foliar presented best results in saline and non-saline fields. Salt sensitive cultivar EV 1089 clearly showed poor growth as no biomass yield was obtained at control and foliar Si application at twelve leaf stage under saline condition.

For future suggestions, more field experiments in salt affected soils are required. Foliar Si application is a viable strategy in field conditions to combat salt toxicity so it should be adopted on large scale. Salt tolerant cultivar Syngenta 8441 showed excellent growth in salt stress condition so this cultivar should be grown on salt affected area.

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